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ABSTRACT

The report includes

Presentations at the Research Planning Workshop on Health Effects of Oxidants (Raleigh, North Carolina, January 28-30, 1980) are documented. The participants include scientists, administrators, and regulatory personnel from the following agencies and institutions: U.S. Environmental Protection Agency, Brookhaven National Laboratory, Oak Ridge National Laboratory, Lawrence Berkeley Laboratory, Inhalation Toxicology Research Institute, EPA Science Advisory Board, California Air Resources Board, University of California - Los Angeles, University of Southern California, University of California - Davis, University of California - Santa Barbara, and University of Rochester.

The focus of the entire volume is the EPA-funded research that is planned or in progress under Theme 1 ("Health Effects of Criteria and Non-Criteria Pollutants from Fossil Fuel Combustion") of the Energy Interagency Health and Ecological Effects Program. The relevance of component projects to EPA regulatory activities is a frequent topic of informal discussion.

Chapter topics fall into five general categories: regulatory activities and concerns, general overviews of research programs, specific animal studies, specific human studies, and biomathematical modeling. Regulatory chapters

review the legislative framework within which EPA operates and suggest lines of research relevant to regulatory needs. General program overviews are presented for each of the agencies and institutions represented.

Approximately half of the total 40 chapters describe animal experimentation. Most of the reported studies involve inhalation exposures to ozone, nitrogen dioxide, or sulfur dioxide followed by examination of biochemical, morphometric, or functional parameters. *In vitro* methods receive some attention.

With respect to human studies, both epidemiologic and experimental projects are reported. The experimental protocols involve inhalation exposures to ozone or nitrogen dioxide followed by measurements of pulmonary function. Chapters on biomathematical modeling discuss projections of human absolute dosage and of the health effects of energy-related air pollution.

The final chapter is a transcript of the Panel Discussion which concluded the workshop. The issues raised involve formal review of EPA-funded research for scientific merit and relevance to regulatory activities.

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ABBREVIATIONS

Ab's -- antibodies
AChE -- acetylcholinesterase
APH -- acetylphenyl hydrazine
ARB -- Air Resources Board
BHT -- butylated hydroxytoluene
BP -- benzopyrene
CASAC -- Clean Air Scientific Advisory Committee
CEQ -- Council on Environmental Quality
COMT -- catechol O methyltransferase
DEE -- Department of Energy and Environment
DEN -- diethylnitrosamine
DMBA -- dimethylbenzanthracene
DNA -- deoxyribonucleic acid
DOE -- Department of Energy
2,3-DPG -- 2,3-diphosphoglycerate
ECAO -- Environmental Criteria and Assessement Office
FEV_1 -- forced expiratory volume
FIV -- forced inspiratory volume
FRC -- functional residual capacity
FVC -- forced vital capacity
FWS -- Fish and Wildlife Service
FY -- fiscal year
GPx -- glutathione peroxidase
GRase -- glutathione reductase
GSH -- reduced glutathione
G6PD -- glucose-6-phosphate dehydrogenase
Hb -- hemoglobin
Hct -- hematocrit
HRP -- horseradish peroxidase
ITB -- Inhalation Toxicology Branch
IVPL -- isolated ventilated perfused lung
LALN -- lung-associated lymph nodes
LC_{50} -- median lethal concentration
LDH -- lactate dehydrogenase
MAA -- macroaggregated albumin
MAO -- monoamine oxidase
MDH -- malate dehydrogenase
MetHb -- methemoglobin
MMAD -- mass median aerometric diameter

MMEF -- maximal midexpiratory flow rate
MVV -- maximal voluntary ventilation
NAAQS -- National Ambient Air Quality Standards
NAS -- National Academy of Science
NASA -- National Aeronautics and Space Administration
NBS -- National Bureau of Standards
NIEHS -- National Institute of Environmental Health Sciences
NIH -- National Institutes of Health
NIOSH -- National Institute of Occupational Safety and Health
NMU -- N-methyl-N-nitrosourea
NOAA -- National Oceanic and Atmospheric Administration
NOEL -- "no observed effects" level
NPSH -- nonprotein sulfhydryl
OAQPS -- Office of Air Quality Planning and Standards
OMB -- Office of Management and Budget
ORD -- Office of Research and Development
ORNL -- Oak Ridge National Laboratory
PCH -- polycyclic hydrocarbons
pdf -- probability density function
PFT -- pulmonary function test
PK -- pyruvate kinase
PMN -- polymorphonuclear
RAW -- airway resistance
RBC's -- red blood cells
RTP -- Research Triangle Park
SAB -- Science Advisory Board
S.D. -- standard deviation
SOD -- superoxide dismutase
SRAW -- specific airway response
TBA -- thiobarbituric acid reactive products
TGV -- thoracic gas volume
TIC -- trypsin inhibitory capacity
TLC -- thin layer chromatography
TVA -- Tennessee Valley Authority
UCD -- University of California - Davis
UCI -- University of California - Irvine
UCLA -- University of California - Los Angel[illegible]
USDA -- U.S. Department of Agriculture
USGS -- U.S. Geological Survey
UV -- ultraviolet
VC -- vital capacity
3MF -- 3-methylfuran
6PGD -- 6-phosphoglycerate dehydrogenase

1. INTRODUCTION

Robert E. Lee

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On January 28-30, 1980, the Health Effects Research Laboratory (Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina) sponsored a Research Planning Workshop on Health Effects of Oxidants. The meeting was held in Raleigh, North Carolina and was attended by representatives of several governmental and private agencies and institutions.

The workshop focused on the research projects that are planned or in progress under Theme 1 ("Health Effects of Criteria and Non-Criteria Pollutants from Fossil Fuel Combustion") of the Energy Interagency Health and Ecological Effects Program. Over the three days, attendees heard approximately 30 presentations on these efforts. Also presented were the views of EPA personnel who will use the results of such research in the regulatory process. The purpose of the present volume is to document each presentation and the often lively discussion that followed.

The subsequent chapters underscore the fact that research is a very human activity. In addition to the essential ingredients of money, facilities, and instrumentation, it is still the individual researcher's creativity, dedication, and often luck that may mean the difference between successful, reproducible results and failure. The individual researcher will always be the key to successful research. Accordingly, the basic workshop goals were to encourage the exchange of knowledge, understanding, and interpretation of oxidants health effects research and, most importantly, to provide a forum for individual creativity, ideas, and perspective on the additional research needed to develop the data base that is essential for protecting the public health. To that end, the workshop was a notable success.

2. PROGRAM PLANNING FOR ENERGY HEALTH EFFECTS FROM CRITERIA AND NON-CRITERIA POLLUTANTS

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U.S. Environmental Protection Agency
Washington, DC 20460

ENERGY INTERAGENCY HEALTH AND ECOLOGICAL EFFECTS PROGRAM

Background: The First Five Years

The Energy Interagency Health and Ecological Effects Program involves coordination and funding of energy-related research by EPA and 10 other federal agencies. The program was created in 1973 after the Arab oil embargo emphasized the impending acceleration of our country's energy development and the need to address resultant environmental and health concerns. Creation of the program was driven by what is commonly known as the "King-Muir Report" written by Don King of the State Department and Warren Muir of the Council on Environmental Quality (CEQ). With the assistance of Dr. Steven Gage and personnel from about 20 federal agencies, King and Muir assembled an initial five-year energy program that involved EPA and 10 other agencies. The Office of Management and Budget (OMB) and CEQ approved the program for a five-year period to begin with fiscal year (FY) 1975 and end with FY 1979.

Table 2-1 indicates the basic program that remained in place for the first five years. The same agencies continue to participate. An important distinction is that research at the National Laboratories, unlike research at the listed agencies, is not funded on a "pass through" basis. ("Pass through" refers to the procedure by which funds appropriated to EPA are "passed through" to other agencies.) In the case of the National Laboratories, existing projects were transferred to EPA.

Recent Planning: The Second Five Years

About 18 months ago, OMB and CEQ approved the continuation of the program for a second five years. At that time, those of us involved in program management decided to evaluate what program changes, if any, should be implemented. Two major criteria for program development in the second five years were identified: relevancy to agency programmatic needs and a client relationship. The first of these refers not only to EPA needs but also to the needs of the other participating pass through agencies. The second criterion stems in part from frequent criticism that EPA research lacks a "client" or "customer." We in EPA have become very cognizant of this criticism and have found that, in order to survive the budget cycles, we need to have a client who wants the research, who works with us throughout development and conduct of the research, and who receives the output of the research.

With respect to the sources of research requirements, our first goal was to respond to national energy needs and problems. We negotiated with other

TABLE 2-1. ENERGY INTERAGENCY HEALTH AND ECOLOGICAL EFFECTS PROGRAM: PARTICIPANTS AND MAJOR PROGRAM AREAS FOR THE FIRST FIVE YEARS

	Program Area			
Agency[a]	Ecological Effects	Air Transport and Transformation	Measurement and Instrumentation	Health Effects
EPA	X	X	X	X
National Laboratories	X	X		X
DOE	X		X	X
FWS	X			
NBS			X	
NOAA	X	X	X	
TVA	X	X	X	
NIOSH			X	X
NASA			X	
USGS			X	
USDA	X			
NIEHS				X

[a] Definitions of agency abbreviations: DOE, Department of Energy; FWS, Fish and Wildlife Service; NBS, National Bureau of Standards; NOAA, National Oceanic and Atmospheric Administration; TVA, Tennessee Valley Authority; NIOSH, National Institute of Occupational Safety and Health; NASA, National Aeronautics and Space Administration; USGS, U.S. Geological Survey; USDA, U.S. Department of Agriculture; NIEHS, National Institute of Environmental Health Sciences.

agencies to ensure sufficient emphasis in such areas as acid rain, synthetic fuels, air transport, visibility, and complex terrain.

Of course, consideration of EPA programmatic needs was also extremely important in the planning effort. About three years ago, Dr. Gage set up four pilot Research Committees (Mobile Sources, Drinking Water, Pesticides, and Gaseous and Inhalable Particulates). In these Committees he brought together staff members of EPA program offices and researchers working for the Office of Research and Development (ORD). The objective was to jointly plan the ORD program, to assure its responsiveness to program office needs.

This Research Committee system has worked extremely well: there are 12 Committees for FY 1980. With very minor exceptions, the whole ORD program is strongly influenced by the Committees. From the researchers' point of view, the Committees provide the advantage of close coordination with the program offices (the "clients"). Researchers have a forum in which to express their own particular concerns (e.g., the need for longer-term research). On the other side of the fence, the advantage to the regulatory offices is clear: they have a strong voice in deciding what research will be performed.

As a third source of research requirements, we considered the needs of the other participating agencies. The whole character of the interagency program is based on negotiation of research projects that have high priority in terms of mutual concern. For example, DOE is extremely concerned about synthetic fuels; so is EPA. Our challenge in negotiating such programs is to identify the particular projects which are most relevant to both agencies. The system forces and fosters a good working relationship between EPA and the other participating agencies.

HEALTH EFFECTS PROGRAM AREA

Table 2-2 outlines the Health Effects program area for FY 1980. This research effort is divided into a total of six "Themes"; funding totals ~$18 million. Theme 1, Health Effects from Criteria and Non-Criteria Pollutants from Fossil Fuel Combustion, has so far been the major focus of our FY 1981 planning exercise (discussed below).

Theme 3, Fossil Fuel Leachate Hazards, refers to the leaching of materials from fossil fuel combustion into waters and soils. The main EPA clients for this work are the regulatory personnel concerned with drinking water.

Theme 4, Toxicological Studies of Specific Energy-Related Agents, consists of research on cadmium performed at the Comparative Animals Research Laboratory at Oak Ridge National Laboratory. Originally, this Theme involved four or five compounds, but we subsequently discovered that most of the other agents are covered by ORD base program activities.

Theme 5, Relative Risk Assessments for Specific Energy Systems, seeks to compare the known risks of conventional combustion with those of the newer

TABLE 2-2. HEALTH EFFECTS PROGRAM AREA:
THEMES, PARTICIPANTS, AND FUNDING LEVELS FOR FISCAL YEAR 1980

	Funding (thousands of dollars)[a]						
Theme	HERL-RTP	HERL-CIN	DOE_x	DOE	NIEHS	NIOSH	Total by Theme
1. Health Effects of Criteria and Non-Criteria Pollutants from Fossil Fuel Combustion	2050		2368		1150		5568
2. Development and Validation of Bioassay Screens and Predictor Test Protocols		500	4065		2150		7215
3. Fossil Fuel Leachate Hazards		600					600
4. Toxicological Studies of Specific Energy-Related Agents (Cadmium)			775				775
5. Relative Risk Assessments for Specific Energy Systems (Fluidized Bed Combustion Versus Conventional Combustion)	400				500	1912	2312
6. Advanced Fossil Fuel Cycle Hazards				1217		300	1517
Total by Agency:	2450	1100	7208	1217	3800	2212	17987

[a] Definitions of agency abbreviations: HERL-RTP, Health Effects Research Laboratory (EPA-ORD), Research Triangle Park, NC; HERL-CIN, Health Effects Research Laboratory (EPA-ORD), Cincinnati, OH; DOE_x, Department of Energy Projects transferred to EPA; DOE, Department of Energy; NIEHS, National Institute of Environmental Health Sciences; NIOSH, National Institute of Occupational Safety and Health.

fluidized bed combustion. The National Institute of Occupational Safety and Health has a major role in this Theme. There are ~14 epidemiologic projects focusing on workplace environments.

Planning for 1980: Phase I

One of the first steps in our planning exercise for FY 1980 was to discuss with the EPA program offices their energy-related research requirements. Walter Barber of the Office of Air Quality Planning and Standards (OAQPS) indicated a primary need for additional health effects studies to support review and revision of National Ambient Air Quality Standards for both Criteria and Non-Criteria Pollutants. Barber also stressed the need for additional epidemiologic and clinical studies of human health. His reason for this request was that he finds human data much more convincing to Congress and to the public interest groups. These groups are not easily convinced of the relevance of animal toxicological data in establishing or revising standards. Barber was careful to point out that he recognizes the importance of animal data, and only advocates more of a balance between human and animal studies.

For the Energy Health Effects program, at least, Barber's was a valid criticism: at that time we had no epidemiology and a fairly low level of clinical work. We responded by dedicating almost half of the 1980 Energy Health Effects program to epidemiology and clinical work. We still have a large animal toxicology program (as does the ORD base program). The key question of what constitutes an "appropriate balance" between human and animal work is a difficult one for which there may be no definitive answer.

Following these discussions with OAQPS, the Energy Health Effects program was structured into the six Themes shown in Table 2-2. Theme 1 consists partly of animal inhalation toxicology projects inherited from the National Laboratories via an OMB mandate that $14 million worth of existing projects in the health/ecological effects area be transferred to EPA. The intent of the transfer was to broaden EPA capabilities in this area by allowing us to draw upon the tremendous talent and expertise residing in the National Laboratories. At the same time, ~$14 million worth of control technology work was transferred from EPA to DOE. OMB attached several ground rules to the transfer: Only projects involving conventional combustion would be transferred to EPA; no human work would be affected. Also, no project changes would take place in FY 1979 and 1980. The two-year moratorium on project changes gave those of us in EPA a welcome opportunity to deal with our own heavy workload and to become better acquainted with the National Laboratories investigators.

With respect to any changes for FY 1981, we prefer to keep project resources within the particular National Laboratory in which they reside, and we are absolutely committed to maintaining the total resources within the National Laboratory system. Thus, we might shift a particular project from Brookhaven to Oak Ridge, for example, but we will not take those project resources out of the National Laboratory system.

Also included in Theme 1 are some clinical projects carried over from the first five years, as well as some new epidemiologic work.

Planning for 1981: Phase II

About six months ago we embarked upon Phase II of planning for the Energy Health Effects program. As it would have been impossible to perform an in-depth evaluation of the entire $18-million program, our strategy was to focus on that part of the program which responds to OAQPS needs. With the assistance of Richard Dowd we assembled a small Science Advisory Board (SAB) subcommittee to assist us in this effort. Dr. James L. Whittenberger was named Chairman of the subcommittee (see Chapter 3 of this volume).

Our first public meeting was held in Washington on November 13-14, 1979. This was an "information meeting": no particular advice was requested or received. Rather, the intent was to explain our general planning philosophy and procedure. The meeting agenda was split into three major areas. First, OAQPS staff described their research requirements in as much detail as possible. Secondly, ORD staff presented the ORD base program. In this, our goal was to promote coordination and minimum overlap between the Energy Health Effects program and the base program (which has been in existence for ~15 years). The final presentation was on the Energy Health Effects program itself. After outlining the program as it stood at that time, we asked the SAB subcommittee to work with us over the next few meetings to identify changes needed to make the program more responsive to OAQPS needs and to ensure better coordination with the ORD base program.

Subsequent to this meeting, we developed what is called the "straw man" document (Miller et al. 1979). The straw man document proposes changes in the Energy Health Effects program to enhance its responsiveness to regulatory needs and improve coordination with the ORD base program.

A second public meeting was held in Washington on December 18-19, 1979. The entire two days were devoted to discussions of the straw man document. The SAB subcommittee concurred with most of the recommendations contained in that document and added recommendations of its own.

In January 1980, Dr. Charles Nauman, our health expert in the Energy Effects Division, and this author made a quick "round robin" trip to the National Laboratories that would be affected by the proposed changes. We discussed the impact of the changes and received their feedback. In several cases we discovered misinterpretations, on our part, of their work; thus, the discussions were of considerable benefit.

In our visits to the National Laboratories, we repeatedly encountered concern about the sometimes short-range nature of EPA programs. With regard to Theme 1, it is significant that the Clean Air Act requires standard revisions and reevaluations on a five-year recurring basis. Walter Barber of OAQPS has stressed the importance that Theme 1 programs continue for five-year periods, to permit acquisition of the best information to make regulatory decisions. And, indeed, we have endeavored to structure the Theme 1 projects on five-year recurring bases.

Aside from the needs of EPA (OAQPS), our Phase II planning exercise has also considered the needs of the other agency that participates in Theme 1: the National Institute for Environmental Health Sciences (NIEHS). NIEHS has expressed concern that the Theme 1 research program gives too much attention to EPA regulatory needs. Clearly, we must work closely with NIEHS to ensure a program consisting of projects that are of high priority to both agencies.

Following the Research Planning Workshop on Health Effects of Oxidants (January 28-30, 1980; documented by this volume), our next step will be to meet again with some of the National Laboratories investigators. A revised straw man document will be published. The SAB subcommittee will hold another public meeting on March 11-12, 1980, at which we will present the final or semifinal set of proposed changes. We hope to complete the final 1981 program decisions in April or May of 1980.

Implementation and Management

Aside from the planning exercise and the resultant program changes, an equally important question is how these changes will be implemented and the entire Energy Health Effects program managed. With respect to management by the Energy Effects Division, our very small staff represents a major constraint: it is not possible to run an $18-million program with one or two individuals. In addition, now that the Energy Health Effects program is more closely coordinated with the ORD base program, it is increasingly apparent that these efforts mesh and that most of the technical knowledge (for Theme 1, at least) exists within ORD at Research Triangle Park, North Carolina (RTP). Thus, our current plans are to assign project officers from the Health Effects Research Laboratory at ORD-RTP where possible.

CONCLUDING REMARKS

A similar planning effort is being mounted for Theme 2 (a ~$7.5 million research effort). Dr. Alan Moghissi from Dr. Gage's office will lead this planning exercise. Once again we have requested SAB's assistance. To provide continuity, we have asked Dr. Whittenberger to chair the Theme 2 SAB subcommittee. Of course, the subcommittee membership will be different (a different expertise is needed).

Major program changes are always difficult, and the Theme 1 planning exercise could not have proceeded this far without tremendous cooperation from all of the participants: the ORD base program, SAB, and particularly the National Laboratories. We appreciate the feedback we have received, and we will continue our efforts to acknowledge that feedback in proposals for program revisions. With everyone's help, the Theme 1 planning exercise will be drawn to a successful conclusion.

REFERENCE

Miller, F. J., J. A. Graham, M. Hazucha, C. Hayes, and W. Riggan. 1979. Preliminary Relevance Review of EPA-DOE Projects on Health Effects of Criteria and Non-Criteria Pollutants from Fossil Fuel Combustion (Theme 1). Unpublished document, Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, December 12.

WORKSHOP COMMENTARY

D. C. Borg: As someone at one of the National Labs, I'd like to ask if there is any intention to involve us in the "takeoff" as well as the "landing." Will you involve us in the planning exercise or will you just tell us its results?

W. Frietsch: Yes, we do plan to involve you in the "takeoff." In fact, we plan to hold a workshop in February 1980 at which all Theme 2 participants will give verbal presentations. Also present will be representatives of our base program and (if assembled) the SAB subcommittee.

As we go through this planning exercise, we're learning that it would be very desirable to involve you more in the "up front" end of it. That's exactly our intent in having a workshop to kick off the whole planning exercise. We will also require (at the meeting) some written material, as we did in the first Theme. But we definitely want people to give verbal presentations and answer questions, etc. about their work.

3. ROLE OF THE SCIENCE ADVISORY BOARD IN RESEARCH PLANNING AND REVIEW

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INTRODUCTION

This report briefly describes the role of the Science Advisory Board (SAB) in the ongoing revisions to Theme 1 of the Energy Health Effects program (see Chapter 2 of this volume). It is important to bear in mind that SAB's role is advisory: we are assisting EPA in redirecting Theme 1 to make it more responsive to the needs of the Office of Air Quality Planning and Standards and to achieve a better balance among the components of relevant animal toxicological, clinical, and epidemiologic research.

Having participated in the review of the criteria document leading to revision of the National Ambient Air Quality Standard for photochemical oxidants, and having participated in the Health Effects Research Review Group which, at the request of Congress, examined health effects research in relation to various EPA programmatic needs, this author feels very strongly about the importance of providing EPA regulatory personnel with the very best possible data. Those who work with criteria documents and who consider the problem of translating scientific information (particularly health effects information) into standards realize that, among the large number of studies cited in a given criteria document, usually only a very small number are really appropriate for standard-setting. It is thus extremely important that those few studies be of high quality. Making such assessments of scientific merit is perhaps the most difficult problem in planning research.

COMMITTEE MAKEUP AND ORIENTATION

SAB participation in the Theme 1 planning exercise dates from September 12, 1979, when Mr. Frietsch and I met to discuss SAB's role. The SAB subcommittee assembled as a result of that meeting consists of Dr. Jack Hackney, Dr. Ian Higgins, Dr. Daniel Menzel, Dr. Richard Riley, and myself as Chairman.

Most of the subcommittee members have a background in reviewing research protocols as study section members; thus, we prefer to know a lot about each project, including the methods to be used, the techniques of data analysis and interpretation, etc. Accordingly, in the Theme 1 exercise, assessing scientific merit has remained (for us, at least) an essential part of our job even though the official subcommittee charge relates to research planning rather than evaluation. Because of this orientation, we have experienced a number of problems in reviewing both the written material given to us as well as presentations at the meetings we have attended. For example, we understand that there will be two new epidemiologic studies: one in El Paso and one in the Southern California Air Basin. We have not been able to find out much about either of these studies; therefore, we feel we can express no judgment whatsoever about them.

STEPS IN THE REVIEW PROCESS

Between the November 13-14 and December 18-19 (1979) public meetings (see Chapter 2 of this volume), EPA conducted its own review of the Theme 1 projects. The EPA staff members involved in this exercise were Dr. Fred Miller, Dr. Judith Graham, Dr. Milan Hazucha, Dr. Carl Hayes, and Dr. Wilson Riggan. These EPA scientists understood that they were to make a relevance review rather than a scientific merit review. Personally, I question whether scientific merit can be separated from relevance. All too often I have heard that a particular study is subject to various significant criticisms and may be of doubtful validity, but "cannot be ignored in standard-setting." My view is: Pending replication, such a study not only can be but should be ignored in standard-setting.

At the December 18-19 meeting our subcommittee received the EPA staff report (Miller et al. 1979), which included both general recommendations and specific recommendations on individual projects. In general, the subcommittee agreed with these recommendations. We could not determine, however, the extent to which the various projects had undergone peer review on an individual basis. We knew that the National Laboratories employ laboratory reviews (program reviews); we could not ascertain the extent to which they employ project reviews.

RECOMMENDATIONS

Following the December meeting, the SAB subcommittee assembled our own tentative recommendations which we consider to be consistent with the EPA recommendations. First, we recommend that rigorous external as well as internal project review be undertaken for fiscal year 1981 and thereafter. We strongly endorse the exchange between investigators of protocols for clinical research. We find certain projects to be of low priority (at least, as EPA priorities have been defined to us), and we recommend a major reprogramming of effort. Redirection of Theme 1 projects can lead, we believe, to an increase in clinical and epidemiologic work. At present, our subcommittee lacks sufficient information to determine what that balance should be. One of the principal goals of future meetings will be to obtain such information.

CONCLUDING REMARKS

With regard to comments by Frietsch (Chapter 2 of this volume) concerning the needs of other participating agencies, it is not clear why the needs of any agency other than EPA should be of primary concern. In this exercise it is EPA requirements that are at issue. EPA operates under laws that require its research to be relevant to standard-setting and other forms of regulation; if that isn't the case (regardless of whether the studies are long-term or short-term), the research is simply not appropriate for EPA's purposes. We in the SAB subcommittee view the Research Planning Workshop on Health Effects of Oxidants (January 28-30, 1980; documented by this volume) as an opportunity to become much better informed about the individual projects that comprise Theme 1 of the Energy Health Effects program.

REFERENCE

Miller, F. J., J. A. Graham, M. Hazucha, C. Hayes, and W. Riggan. 1979. Preliminary Relevance Review of EPA-DOE Projects on Health Effects of Criteria and Non-Criteria Pollutants from Fossil Fuel Combustion (Theme 1). Unpublished document, Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, December 12.

4. OVERVIEW OF REGULATORY AND COMPLIANCE REQUIREMENTS

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INTRODUCTION AND OVERVIEW

This report attempts to provide some insight into the regulatory process and into how EPA's Office of Air Quality Planning and Standards (OAQPS), as a customer for the output of the scientific community, uses information generated by scientists in developing standards and regulations. The key point, perhaps, is that setting a standard is not strictly a scientific enterprise. Certainly at the heart of a standard's development is the scientific information upon which a choice must be based. It is important to recognize, however, that there are other ingredients in setting National Ambient Air Quality Standards (NAAQS) (or any other standards, for that matter).

Interpretation of the Clean Air Act and the legislative history is not an activity of OAQPS alone. We rely on EPA's Office of General Counsel and their attorneys to provide us with information on how far to go (if at all) in considering economics, for example, when selecting a standard. Do we consider the issue of attainability? Although we're on record on most of these issues, we nonetheless must rely on the guidance of the Office of General Counsel and their attorneys to give us the proper direction. As an example, probably the key procedural issue during review of the photochemical oxidants standard was the so-called "Shy Panel" that was convened early in the regulatory process. Critics argued that procedural irregularities were associated with that meeting. This controversy flags the importance of working closely with our attorneys to ensure that our actions are proper and in accordance with the law.

There is, of course, the obvious issue of interpretation of the health evidence. This is certainly the area where we spend most of our time and where we need the most communication with the scientific community.

Control effectiveness is another scientific element in development of a standard. In the case of ozone, for example, atmospheric chemistry bears importantly on the effectiveness of control options.

Determining the numbers of persons at risk is yet another scientific enterprise. This information is of interest to the Administrator in choosing the level of a standard.

An understanding of economic impacts is also important, even if that information is not used in setting a standard level. While it is our interpretation of the Clean Air Act that economics not be considered in setting NAAQS, it is also our position that economic impacts be estimated and made clear to the public so that the electorate can, if it sees fit, act through elected officials to modify the way the law is structured. In other words, if the ozone standard is set at 0.12 ppm and provides a certain amount of protection, it is important that the public realize the economic cost associated with that protection. If the electorate feels so inclined, it can make choices for new directions. Therefore, we strive to delineate economic impacts as accurately and definitively as possible.

The decisionmaker (the Administrator of EPA) represents the Administration. His task involves a judgmental element and is not, as noted earlier, a strictly scientific enterprise. There is a margin of safety. How that margin of safety is characterized and what magnitude of risk the decisionmaker will accept are important issues, and we must do our best to clearly present the relevant information to him. Concerns in this area include marginal choices on what constitutes an adverse health effect. In the case of ozone, there is certainly disagreement not only among policymakers but also within the scientific community as to what constitutes an adverse effect. It is unlikely that disagreements of this sort will ever be totally resolved. None the less, judgments must be made that reflect our best understanding of science and of the health policy of our elected officials.

REGULATORY OPTIONS UNDER THE CLEAN AIR ACT

NAAQS are not the only regulatory tool available to EPA for control of air pollutants. Section 112 of the Clean Air Act provides for National Emission Standards for Hazardous Air Pollutants; these exist for mercury, beryllium, asbestos, and vinyl chloride. Standards of Performance for New Stationary Sources (Section 111) are technology-based standards that are, in many cases, designed to support NAAQS. Emission Standards for Moving Sources (Section 202) are likewise tied in and support the attainment of NAAQS.

Other options include the regulation of fuels and fuel additives, inspection maintenance programs, and related control strategies. Also, the Emergency Powers clause (Section 303) permits the Administrator to take immediate action in certain situations if there exists significant risk to individuals.

All of these regulatory options are available to the Administrator to control air pollutants. The Clean Air Act provides clear guidance as to how he can and cannot use each option.

DEVELOPMENT OF NATIONAL AMBIENT AIR QUALITY STANDARDS

In the 1970 Clean Air Act, EPA is tasked to list those ubiquitous pollutants which, in the Administrator's judgment, have adverse effects on public health and welfare. Primary standards protect public health; secondary standards protect the public welfare against known or anticipated adverse effects. The second step is to issue criteria documents containing the latest scientific knowledge on identifiable effects of pollutants on public health and welfare. After publication of each criteria document, a standard is proposed. Next is a period of public comment, followed by promulgation. Finally, there is a requirement for periodic review and (where appropriate) revision of each criteria document and standard.

These provisions are changed and updated in the 1977 Clean Air Act Amendments. With regard to NAAQS, the 1977 Amendments set a timetable for review of all the standards by the end of the 1980 calendar year. The Amendments further require (1) review on a five-year basis of all NAAQS, (2) issuance of nitrogen dioxide criteria for a short-term standard, and (3) establishment of a scientific committee to review criteria and standards.

Determination of a primary standard level entails definition of the adverse effect against which the standard is to protect, definition of the population or group which is most sensitive to the particular effect, and evaluation of all existing medical evidence set forth in the criteria document. In addition, outstanding uncertainties must be defined and reasonable provision made for scientific and medical knowledge not yet acquired.

A number of judgmental choices need to be made in conjunction with the scientific community. EPA interprets the Clean Air Act and the legislative history to indicate that Congress has made a policy judgment in favor of prevention of harm and in favor of exercising caution with regard to the public health.

A margin of safety is necessary to protect against hazards which research has yet to define. This is a legal as well as a scientific issue. In my view, a simple absence of evidence does not confirm that safety exists. While this is a very difficult issue, it is nonetheless something that the Administrator must consider in selecting the final level of a standard.

To protect the most sensitive groups in the population, the Administrator must weigh risks to public health at pollutant levels below those convincingly shown to impact health. Once again, assessment of risk to public health is based on scientific evidence but not based on fact alone. In judging what constitutes an adequate margin of safety, uncertainties in the evidence, conflicting evidence, extrapolation, trends of known facts, and projections from imperfect data must all be weighed. We simply cannot ignore incomplete or inconclusive evidence.

Scientific information must be generated and then presented and compiled into a criteria document. Subsequently there is involvement of the scientific community and review groups to ensure the adequacy of that document and that we have the best possible basis upon which to set a standard.

Since the 1977 Amendments, a staff paper has been employed to signal to the public, prior to proposal, the way in which EPA interprets the medical evidence in the criteria document. The staff paper is a public document which is subject to public review. In the carbon monoxide staff paper, we did our best to present to the Clean Air Scientific Advisory Committee (CASAC) of the Science Advisory Board (SAB) how we plan to use the criteria document information in the regulatory process.

The next step is an internal review process within the Agency. Then the Administrator makes a decision on the standard. Following the Administrator's decision, a standard is proposed.

To indicate the length of time involved, our meeting with CASAC(SAB) to discuss the carbon monoxide staff paper was held on June 11, 1979; hopefully we'll be able to propose a standard in February or March of 1980. Development of NAAQS is not a speedy process. In the case of the original oxidants standard, it was probably a 30-month period from the time the scientific information was compiled and presented in the first draft of the criteria document until we proposed or promulgated the standard.

ISSUES IN DEVELOPMENT OF THE OZONE STANDARD

The 1977 Clean Air Act Amendments required EPA to review the 1971 National Ambient Air Quality Standard for Photochemical Oxidants. Today, the revised standard (the "Ozone" standard) is probably the most expensive environmental program in the country, with an estimated $5-10 billion annual cost. Most of that cost is associated with the emission standards on automobiles.

Of course, there was (and is) a wide divergence of medical opinion with regard to the scientific evidence on ozone. Therefore, the scientific community's assistance was essential in providing our Administrator with the kind of information that he needed in order to make a reasoned judgment.

Besides medical questions, a number of other issues surfaced during development of the ozone standard. One such issue was severity of effects. A reinterpretation of evidence from the study upon which the original oxidants standard was based (the Schoettlin/Landau study) led to designation of a new estimated effect level in the revised criteria document. There was a continuing debate with the attorneys as to whether that action was appropriate or inappropriate. Review of the criteria document by CASAC(SAB) pointed up differences of opinion as to the quality of the document.

Another issue centered on measurement methods for singlet oxygen. The State of Texas brought to our attention what it considered to be an important finding: that all the clinical studies were simply invalid due to artifact production by the ozone generators. This issue had to be resolved prior to promulgation of the standard.

The Council of Economic Advisors, after reviewing the standard, suggested that the level could be relaxed above what was proposed. The Council felt that the Federal Government should consider cost across public health and safety fields: For example, are health dollars spent to protect people from ozone as cost-effective as those that could be spent on highway guard rails? We simply don't feel that this is an issue, because the Clean Air Act emphasizes the need for protection of public health from air pollutants.

Other issues included: economics, our modification of the nomenclature from "photochemical oxidants" to "ozone," the contribution of natural ozone, and adequate margins of safety.

CURRENT RESEARCH NEEDS

During development of the ozone standard, we flagged several areas in which a need for additional scientific evidence exists. For example, to help resolve the issue of what constitutes an adverse health effect, we need to improve the characterization of the kind and magnitude of various pulmonary function detriments, biochemical changes, and other responses.

We require a better understanding of the importance of the animal studies, particularly with regard to increased resistance to bacterial infection. Although (to the author's knowledge) this effect has not been demonstrated in clinical studies, we remain concerned due to the seriousness of any such potential effect. Any information on its relevance to human health is certainly needed.

Determination of the long-term chronic effects of photochemical oxidants and of the potential for such damage as accelerated aging of body tissues, chromosomal damage, mutagenesis, carcinogenesis, etc. is another area in which good information is sparse. Often we don't understand the significance or importance of the effects that have been observed.

Identification of particularly sensitive groups and investigation of adverse effects on materials are additional scientific needs.

REFERENCES

Clean Air Act. 42 USC 7401 et seq.

Clean Air Act Amendments of 1977. Public Law 95-95, August 7, 1977.

5. CRITERIA DOCUMENT REVIEW AND REVISION

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INTRODUCTION

The review and revision of criteria documents constitute a broad topic, but may be considered to consist of three aspects: the framework, the procedures, and the content. The framework is made up of the Clean Air Act requirements, the organizational structure of the pertinent EPA organizations, and the division of responsibilities among those organizations. The procedures followed are those stipulated by the Clean Air Act and those established by EPA on the bases of precedent, practice, and practicality. The content of a criteria document is determined by the Clean Air Act, by the nature of the pollutant under consideration, and by the intended use of the document.

THE FRAMEWORK

Section 108 of the Clean Air Act as amended in 1977 specifies that the Administrator issue air quality criteria for each air pollutant

> (a)(1)(A) emissions of which, in his judgment, cause or contribute to air pollution which may reasonably be anticipated to endanger public health or welfare;
>
> (B) the presence of which in the ambient air results from numerous or diverse mobile or stationary sources;...

The Act further specifies that

> (2) ...Air quality criteria for an air pollutant shall accurately reflect the latest scientific knowledge useful in indicating the kind and extent of all identifiable effects on public health or welfare which may be expected from the presence of such pollutant in the ambient air, in varying quantities. The criteria for an air pollutant, to the extent practicable, shall include information on--
>
> (A) those variable factors (including atmospheric conditions) which of themselves or in combination with other factors may alter the effects on public health or welfare of such air pollutant;
>
> (B) the types of air pollutants which, when present in the atmosphere, may interact with such pollutant to produce an adverse effect on public health or welfare; and
>
> (C) any known or anticipated adverse effects on welfare.

It should be noted at the outset that the Clean Air Act does not employ the terminology "criteria document." It specifies the "issuance" of criteria, but does not specify the form or format in which such criteria are to be issued or disseminated. Criteria documents as they presently exist are the product of an evolving process that had its genesis in legislation prior to the Clean Air Act and that was first manifested in the criteria documents issued in 1970. Early legislation did specify the publication of information on the effects of and control of air pollutants, but subsequent legislation did not refer specifically to publication of criteria.

Not only does it not specify the form in which criteria are to be issued, the Clean Air Act also does not define "air quality criteria." By dictionary definition, criteria are "rules, tests, or indices that can be used as a basis for decisions or judgments." It is clear from the portion of Section 108 cited above that criteria are to be descriptions of the effects of air pollutants on man and his environment. Combining the dictionary definition with the definition implicit in Section 108, "air quality criteria" are descriptions of air pollution effects presented in a manner that renders them suitable as the scientific basis for National Ambient Air Quality Standards (NAAQS). Thus, in practice, air quality criteria are descriptions of cause-and-effect relationships expressed, to the extent possible, as dose-response functions. Criteria documents, then, while they may serve a number of worthwhile purposes, are prepared primarily as the vehicle for issuance of the criteria that serve as the scientific basis for NAAQS. Air quality criteria, as indicated in Section 108, must reflect the latest scientific information available that pertains to the effects of an air pollutant, direct or indirect, on man and his environment. Scientific information needed to formulate control strategies--such as that useful in defining atmospheric transformations and source-receptor relationships--is to be included only to the extent practicable.

The legislative framework that determines the basic philosophy and content of criteria documents has been discussed above. A brief examination follows of the organizational framework within which document review and revision proceed.

Responsibility for the preparation of air quality criteria documents rests with the Environmental Criteria and Assessment Office (ECAO). Located at Research Triangle Park, North Carolina, ECAO is one of two such offices within the Office of Health and Environmental Assessment; the latter is in turn part of the Office of Research and Development (ORD) (Figure 5-1). Responsibility for deriving and promulgating NAAQS, and for issuing control techniques information, lies with the Office of Air Quality Planning and Standards (OAQPS), which is part of the Office of Air, Noise and Radiation. Because the Clean Air Act stipulates that standards be proposed and information on control techniques be issued at the same time that criteria are issued, close coordination between ECAO and OAQPS is required. Most day-to-day communication occurs directly between these offices.

THE PROCEDURES

The responsibilities of ECAO versus those of OAQPS in the review and revision of criteria and standards are shown in Figure 5-2. Segments in which

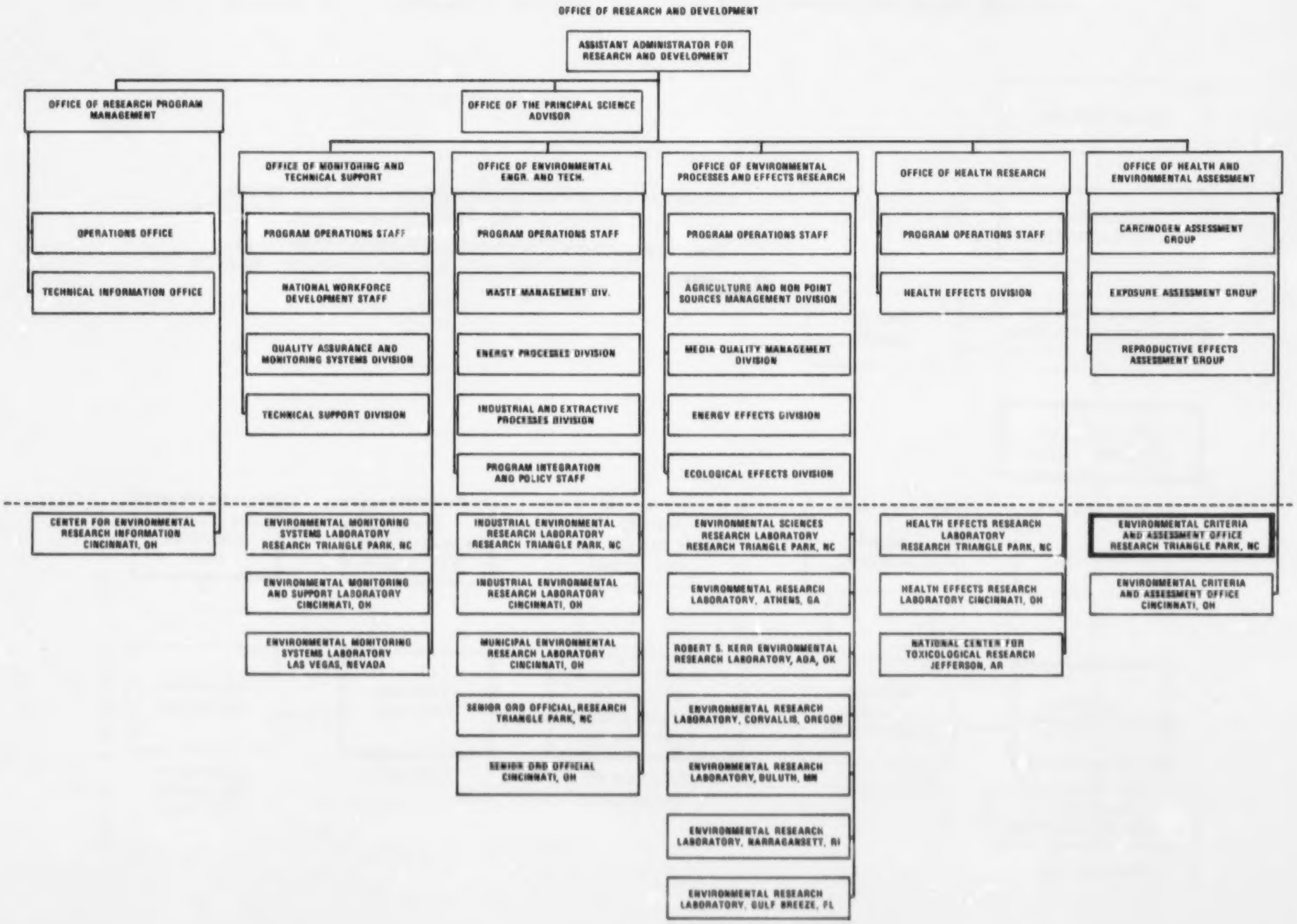
OFFICE OF RESEARCH AND DEVELOPMENT
ASSISTANT ADMINISTRATOR FOR RESEARCH AND DEVELOPMENT
OFFICE OF RESEARCH PROGRAM MANAGEMENT
OFFICE OF THE PRINCIPAL SCIENCE ADVISOR
OFFICE OF MONITORING AND TECHNICAL SUPPORT
OFFICE OF ENVIRONMENTAL ENGR. AND TECH.
OFFICE OF ENVIRONMENTAL PROCESSES AND EFFECTS RESEARCH
OFFICE OF HEALTH RESEARCH
OFFICE OF HEALTH AND ENVIRONMENTAL ASSESSMENT
OPERATIONS OFFICE
TECHNICAL INFORMATION OFFICE
PROGRAM OPERATIONS STAFF
NATIONAL WORKFORCE DEVELOPMENT STAFF
QUALITY ASSURANCE AND MONITORING SYSTEMS DIVISION
TECHNICAL SUPPORT DIVISION
PROGRAM OPERATIONS STAFF
WASTE MANAGEMENT DIV.
ENERGY PROCESSES DIVISION
INDUSTRIAL AND EXTRACTIVE PROCESSES DIVISION
PROGRAM INTEGRATION AND POLICY STAFF
PROGRAM OPERATIONS STAFF
AGRICULTURE AND NON POINT SOURCES MANAGEMENT DIVISION
MEDIA QUALITY MANAGEMENT DIVISION
ENERGY EFFECTS DIVISION
ECOLOGICAL EFFECTS DIVISION
PROGRAM OPERATIONS STAFF
HEALTH EFFECTS DIVISION
CARCINOGEN ASSESSMENT GROUP
EXPOSURE ASSESSMENT GROUP
REPRODUCTIVE EFFECTS ASSESSMENT GROUP
CENTER FOR ENVIRONMENTAL RESEARCH INFORMATION CINCINNATI, OH
ENVIRONMENTAL MONITORING SYSTEMS LABORATORY RESEARCH TRIANGLE PARK, NC
ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY CINCINNATI, OH
ENVIRONMENTAL MONITORING SYSTEMS LABORATORY LAS VEGAS, NEVADA
INDUSTRIAL ENVIRONMENTAL RESEARCH LABORATORY RESEARCH TRIANGLE PARK, NC
INDUSTRIAL ENVIRONMENTAL RESEARCH LABORATORY CINCINNATI, OH
MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY CINCINNATI, OH
SENIOR ORD OFFICIAL, RESEARCH TRIANGLE PARK, NC
SENIOR ORD OFFICIAL CINCINNATI, OH
ENVIRONMENTAL SCIENCES RESEARCH LABORATORY RESEARCH TRIANGLE PARK, NC
ENVIRONMENTAL RESEARCH LABORATORY, ATHENS, GA
ROBERT S. KERR ENVIRONMENTAL RESEARCH LABORATORY, ADA, OK
ENVIRONMENTAL RESEARCH LABORATORY, CORVALLIS, OREGON
ENVIRONMENTAL RESEARCH LABORATORY, DULUTH, MN
ENVIRONMENTAL RESEARCH LABORATORY, NARRAGANSETT, RI
ENVIRONMENTAL RESEARCH LABORATORY, GULF BREEZE, FL
HEALTH EFFECTS RESEARCH LABORATORY RESEARCH TRIANGLE PARK, NC
HEALTH EFFECTS RESEARCH LABORATORY CINCINNATI, OH
NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH JEFFERSON, AR
ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE RESEARCH TRIANGLE PARK, NC
ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE CINCINNATI, OH

Figure 5-1. Office of Research and Development, U.S. Environmental Protection Agency.

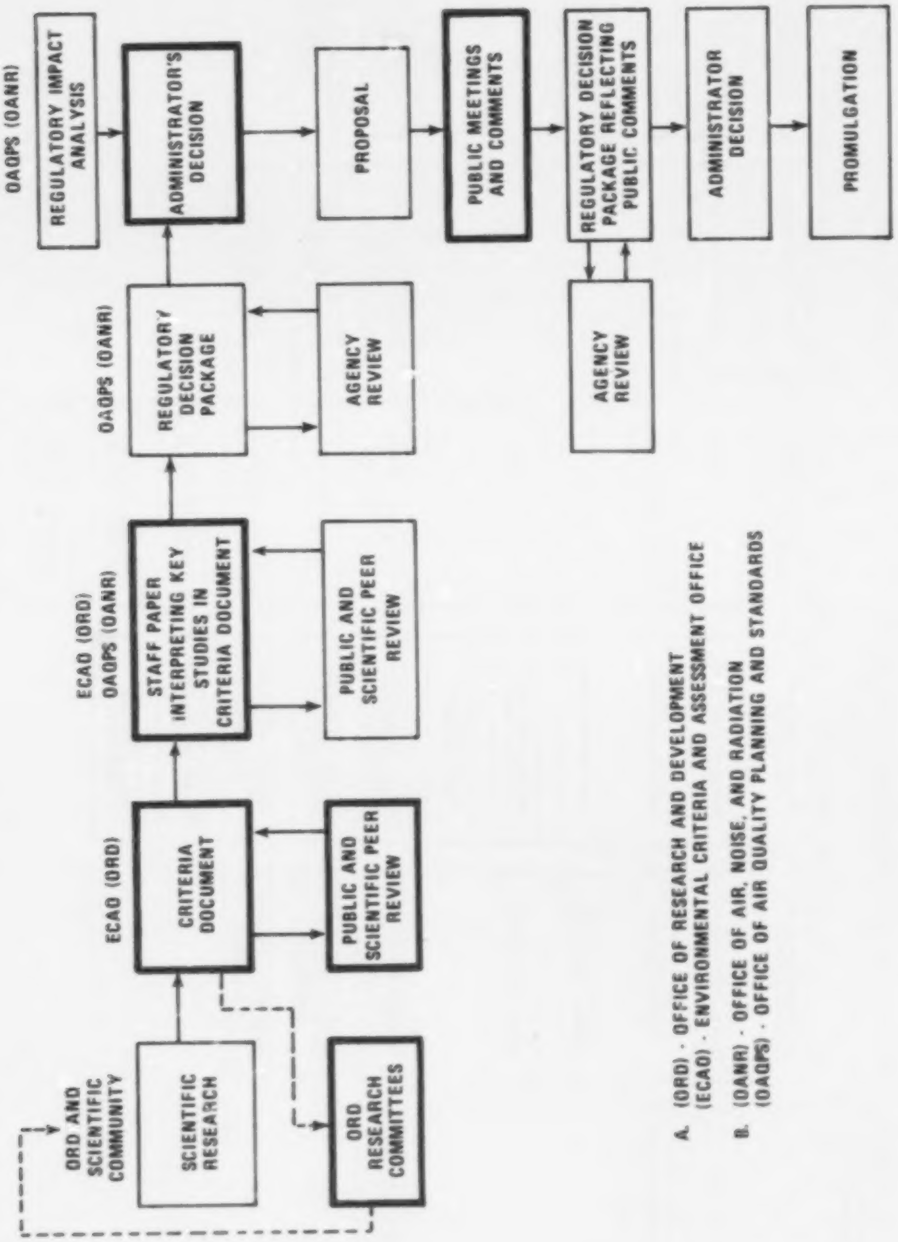
ORD AND SCIENTIFIC COMMUNITY
SCIENTIFIC RESEARCH
ORD RESEARCH COMMITTEES
ECAO (ORD)
CRITERIA DOCUMENT
PUBLIC AND SCIENTIFIC PEER REVIEW
ECAO (ORD) OAQPS (OANR)
STAFF PAPER INTERPRETING KEY STUDIES IN CRITERIA DOCUMENT
PUBLIC AND SCIENTIFIC PEER REVIEW
OAQPS (OANR)
REGULATORY DECISION PACKAGE
AGENCY REVIEW
OAQPS (OANR)
REGULATORY IMPACT ANALYSIS
ADMINISTRATOR'S DECISION
PROPOSAL
PUBLIC MEETINGS AND COMMENTS
REGULATORY DECISION PACKAGE REFLECTING PUBLIC COMMENTS
AGENCY REVIEW
ADMINISTRATOR DECISION
PROMULGATION
A. (ORD) - OFFICE OF RESEARCH AND DEVELOPMENT
(ECAO) - ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
B. (OANR) - OFFICE OF AIR, NOISE, AND RADIATION
(OAQPS) - OFFICE OF AIR QUALITY PLANNING AND STANDARDS

Figure 5-2. National Ambient Air Quality Standard-setting process.

ECAO has primary responsibility or participates are highlighted in bold outline. ECAO has responsibility for preparing the criteria document, for reviewing and incorporating (as needed) comments resulting from the public review process, and for helping OAQPS prepare the staff paper that forms a significant part of the regulatory information developed for NAAQS proposal and subsequent promulgation. In addition, ECAO provides technical assistance to OAQPS by helping brief the Administrator on the scientific data base and its significance and by attending public hearings on the standard to present the EPA viewpoint and to answer questions regarding criteria for the pollutant under consideration.

The recent inclusion of ECAO staff as members of ORD research committees is a significant development. This involvement, along with the principal role played on these committees by OAQPS staff, provides a mechanism for pointing out the research needs and problems identified during criteria and standards development. This important feedback mechanism will help ORD plan its research programs and will assist those EPA offices and personnel responsible for developing timetables and allocating funds. In time, the mechanism will also help those of us in ECAO and OAQPS who are in constant search of the data base needed for standard-setting.

Figure 5-2 does not give an adequate view of the effort that must be expended to prepare a criteria document. A better view of the complexity, time, and demands of the review and revision process is shown in Table 5-1. As indicated, the preparation of a criteria document proceeds in six phases.

TABLE 5-1. PHASES IN CRITERIA DOCUMENT PREPARATION

Phase	Activity	Time (days)
I	document planning and initiation	60-120
II	preparation of working draft	60-200
III	preparation of external review draft (revisions to working draft)	60-120
IV	public and CASAC(SAB)* review of external review draft	60-90
V	preparation of final document (revisions to external review draft)	60-100
VI	closure by CASAC(SAB) on document and publication	60-90
	Total (months):	13-24

*Clean Air Scientific Advisory Committee (CASAC) of the Science Advisory Board (SAB).

Phase I consists of (1) literature search and acquisition; (2) publication of a Federal Register notice to solicit information on the pollutant under consideration; (3) selection of the ECAO project manager and team members; (4) development of a work plan, document outline, and schedule; (5) selection of a task force composed of EPA personnel; (6) recruitment of outside consultants to write the document and additional consultants to review it.

Phase II consists of (1) reading and analyzing literature; (2) continuing the acquisition of literature; (3) writing; (4) holding meetings between the ECAO team and consultants; (5) typing, proofreading, and printing the working draft; and (6) distributing the draft for internal review by EPA personnel and consultants.

Phase III begins with the convening of a workshop, which is one of the best and least time-consuming mechanisms for ensuring that ECAO receives adequate constructive criticism on its documents early in their preparation. Follow-up meetings with consultants are held and the working draft is revised, resulting in an external review draft, the availability of which is announced to the public in a Federal Register notice.

In Phase IV, the document is reviewed by the public, by the Clean Air Scientific Advisory Committee (CASAC) of the Science Advisory Board (SAB), by EPA personnel (including the task force), and by other Federal agencies. The review culminates in presentation and discussion of the external review draft at a public meeting of CASAC(SAB).

Phase V entails assessing comments received (1) by mail from the general public and governmental sectors and (2) at the public meeting from CASAC(SAB) members and other attendees. All comments are logged in, are reviewed, and as appropriate are incorporated in revisions to the document. All comments and responses to those comments are kept as part of the public record for the document.

In the final phase, the revised draft is recirculated to CASAC(SAB) if requested. If major changes have been made to it, the document is also recirculated to the public for a second review and is presented and discussed at a second public meeting of CASAC(SAB). When the document meets its approval, CASAC(SAB) submits a letter of concurrence and a written report to the Administrator of EPA. After further editing for clarity, style, and format, the document is published.

THE CONTENT

The Clean Air Act stipulates that information on the effects of air pollutants on the public health and welfare constitute the minimum and primary content of any air quality criteria document. It recommends, but does not dictate, the inclusion of additional information, as noted earlier in the quotation from Section 108. As possible, or where necessary, information on air quality management is included (e.g., emission inventories, modeling, and atmospheric transport and transformation). Such information is particularly appropriate for secondary pollutants whose source-receptor relationships can only be ascertained through understanding the transformation and transport of the primary pollutants that serve as precursors.

Criteria documents are prepared for substances that have been clearly identified as pervasive pollutants posing threats to public health and the environment. When EPA begins to prepare a criteria document, then, it usually encounters a sizeable and comprehensive body of literature. Typically, an air quality criteria document contains the following:

1. Extensive chapters on animal toxicology, human clinical studies, and human epidemiologic studies, both occupational and nonoccupational. Such chapters include the pertinent information on human metabolism and pharmacokinetics, including uptake, distribution, metabolism or detoxification, and excretion.
2. One or more chapters on the effects of the pollutant on plants, animals, acquatic and terrestrial ecosystems, and natural and man-made materials.
3. One or more chapters that treat fairly exhaustively the methodology available for measuring the pollutant in ambient air, for measuring the pollutant in biological tissues and fluids (where pertinent), and, in a separate chapter or in the health effects chapters, for measuring observed effects. Where possible, these chapters include estimates of the precision, accuracy, and reliability of the methods actually employed to obtain the ambient air, exposure, and effects data presented in the document.
4. Condensed chapters dealing, respectively, with chemical and physical properties; sources and emissions; geographic and temporal distribution of sources; ambient air concentrations; the global cycle of the pollutant in all its forms; and atmospheric transport and transformation of the pollutant.
5. Where possible, a chapter that assesses quantitatively the potential risk for the most sensitive segment of the population.
6. A summary and conclusions chapter focusing on the interpretation and significance of the data cited in the document. This chapter relates ambient air concentrations to observed effects, "no observed effects," and "least observed effects" levels for sensitive or at-risk populations.

As this enumeration indicates, air quality criteria result from the synthesis of essentially two lines of inquiry (Figure 5-3).

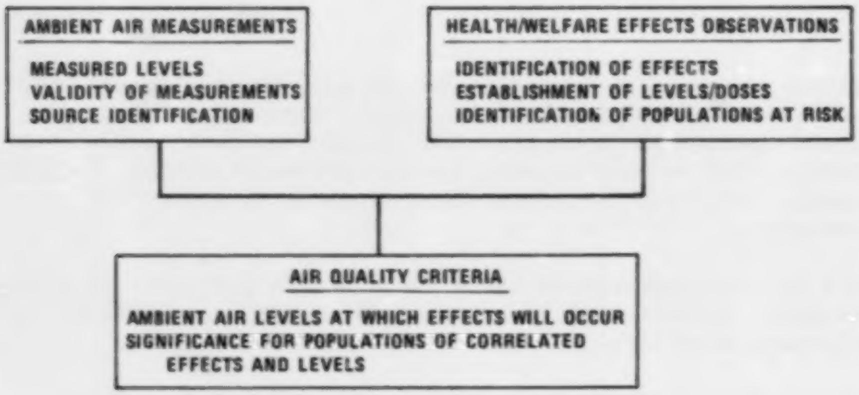
AMBIENT AIR MEASUREMENTS
MEASURED LEVELS
VALIDITY OF MEASUREMENTS
SOURCE IDENTIFICATION
HEALTH/WELFARE EFFECTS OBSERVATIONS
IDENTIFICATION OF EFFECTS
ESTABLISHMENT OF LEVELS/DOSES
IDENTIFICATION OF POPULATIONS AT RISK
AIR QUALITY CRITERIA
AMBIENT AIR LEVELS AT WHICH EFFECTS WILL OCCUR
SIGNIFICANCE FOR POPULATIONS OF CORRELATED EFFECTS AND LEVELS

Figure 5-3. Lines of inquiry resulting in synthesis of air quality criteria.

Without health or welfare effects we would have no criteria upon which to base regulations, but it is equally true that the observation of effects is meaningless in the context of standard-setting if the effects cannot be correlated with the true levels at which they occur. Thus, it is important to be able to measure the criteria pollutant with specificity, accuracy, and precision in the ambient air and in experimental settings. Equally important, though not shown in Figure 5-3, is the ability to measure the effect itself with specificity, accuracy, and precision. Qualitative information on pollutant effects is never disregarded in preparing a criteria document, but it serves mainly to help establish the validity of available quantitative data. To be of use in setting an ambient air standard, air quality criteria should be of a quantitative nature. Air quality criteria, then, are the synthesis of these two basic lines of inquiry and documentation.

It should be noted that air quality criteria must be objective statements of the most recent available scientific evidence. They are to be free from bias, from speculation stated as fact, from economic considerations relative to the protection of public health, and from such language and presentation as might cause them to be construed as standards rather than criteria.

Criteria do not include a margin of safety but, wherever possible, they include an assessment of any uncertainties associated (1) with the severity and nature of the effects caused by the pollutant in question and (2) with the measurement and determination of the concentrations at which such effects occur.

CURRENT RESEARCH NEEDS

No discussion of the review and revision of criteria and criteria documents would be complete without brief mention of areas in which further research and guidance are needed from the scientific community, both within and outside EPA. The following list identifies recurring or continuing problems that EPA encounters in preparing criteria documents:

1. What is a health effect? An adverse health effect?
2. Can we extrapolate from animal studies to human health effects? From clinical studies, with their limited numbers of subjects, to populations? From high-level, short-term exposures to low-level, long-term exposures?
3. Can we establish "no observed effects" levels (NOEL'S)? Are there thresholds? Are there threshold ranges?

4. Have we overlooked important confounding or intervening variables in our epidemiologic studies, such that the reported results are made suspect?

5. Have we overlooked important factors in what may be a multifactorial etiology?

6. How good are our measurements of ambient air concentrations? Of doses administered? Of background levels? Of responses elicited?

7. Does ambient air monitoring of the pollutant in question adequately reflect actual human exposure? That is, can we adequately describe source-receptor relationships?

8. What kinds of groups should be excluded from consideration as sensitive population(s)?

The first question of this list may be unanswerable by scientific inquiry, since part of the answer may be judgmental. For example, the Clean Air Act itself allows the Administrator of EPA to make certain decisions according to his "best judgment." Furthermore, even health researchers may differ about what constitutes a health effect and particularly about what constitutes an adverse health effect. Certain kinds of experiments, however, can at least provide a partial answer to this question. Long-term, low-level exposure experiments plus specific experiments on adaptation may be of value. Such low-level chronic exposures may also help resolve whether thresholds exist and whether the NOEL's we try to identify are real or only a product of the insensitivity of present test systems.

Some of the recurring problems listed here arise during derivation of the standard rather than in preparation of the criteria document itself. The last question, for example, is usually treated in the staff paper that is part of the regulatory package forwarded to the Administrator. If smokers, for example, were excluded from consideration as the sensitive population to be protected by a carbon monoxide primary standard, such an exclusion would be a policy decision rather than a scientific judgment. Writers of a criteria document, however, must try to identify all groups at risk and to provide scientific documentation that will aid OAQPS and the Administrator in determining whether a given group should be considered the sensitive group to be protected, or whether a given group can in fact be protected by an ambient air standard.

Finally, broad areas in which ECAO believes research information is presently inadequate include:

1. Human base-line data defining what is "normal" for certain pulmonary function tests and biochemical indicators.
2. Human health effects data resulting from long-term, low-level exposures to air pollutants, especially gas-phase pollutants.
3. Human studies of good design and execution, especially epidemiologic studies.
4. Studies at the biochemical/mechanistic level for gas-phase pollutants.
5. Correlation of classic pulmonary function tests with underlying biochemical/physiological changes.
6. Biochemistry, biophysics, and pharmacokinetics of cardiovascular and cardiopulmonary systems in general, and of the lung in particular.

In view of the basic problems and questions remaining, and in view of the areas in which good scientific data are still lacking, those of us who daily engage in the writing of air quality criteria and related documents look to the scientific community for the continuation of high-quality research, both in the specific areas mentioned above and in the general pathonomy of air pollution related sickness and disease.

REFERENCES

Clean Air Act. 42 USC 7401 et seq.

Clean Air Act Amendments of 1977. Public Law 95-95, August 7, 1977.

WORKSHOP COMMENTARY

K. M. Schaich: You make recommendations which seem quite at variance with some of the recommendations of [Miller et al. 1979; see Chapter 2 of this volume for details of reference]. Where is the kind of mechanistic information that you seek to fund into your program?

B. E. Tilton: Let me point out that ECAO does not do any funding. I hope that question can be addressed by the appropriate committees, but it may be that the needs I have indicated are not the consensus of others involved.

6. TOXICOLOGICAL RESEARCH STRATEGIES IN RELATION TO EPA REGULATORY NEEDS

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INTRODUCTION

Prior to the Research Planning Workshop on Health Effects of Oxidants, prospective attendees were sent an EPA staff report known as a "straw man" document. This document (Miller et al. 1979) proposes certain revisions in the EPA-funded research program designated as "Theme 1" ("Health Effects of Criteria and Non-Criteria Pollutants from Fossil Fuel Combustion"). (For further information on Theme 1 and the entire Energy Interagency Health and Ecological Effects Program, see Chapter 2 of this volume.) As an "old-fashioned" toxicologist, I felt strong personal dismay in reviewing the recommendations contained in the straw man document. In my opinion, the suggested changes diverge so sharply from toxicological dogma (be it good or bad) that an examination of their merits is warranted.

The EPA statements and/or recommendations that I wish to challenge are:

1. Data from studies involving exposures of >1 ppm ozone (O_3) or >5 ppm nitrogen dioxide (NO_2) are not significant to EPA. Sulfur dioxide particulates of >25 mg/m^3 have no impact and have no significance to EPA.
2. All experiments should be conducted at ambient levels.
3. Negative results are as important to EPA as positive results; environmentally relevant exposure regimens should be used.
4. _In vitro_ studies have essentially no value to EPA.

COMMENTS ON EPA RECOMMENDATIONS

In my opinion, to perform studies at ambient levels and to expect other than negative data is to concede that control programs have failed. Obviously, control programs are designed to incorporate margins of safety (see Chapter 4 of this volume).

Negative studies, in my view, have very little value except in completing the bottom ends of dose-response curves that are already well developed. Standard toxicological practice is to range-find _first_, and to then work down to the bottom of the dose-response curve. As a researcher, I do not pursue negative studies because their results are trivial to users.

To me, the fourth statement listed above (regarding _in vitro_ studies) is especially strange. Clearly, EPA needs _in vitro_ studies in order to know what to study _in vivo_! Due to limited resources of time and money, it is simply not possible to perform every study in the whole animal. Results from the relatively fast and inexpensive in vitro systems provide important input in designing fruitful in vivo experimentation.

REFERENCE

Miller, F. J., J. A. Graham, M. Hazucha, C. Hayes, and W. Riggan. 1979. Preliminary Relevance Review of EPA-DOE Projects on Health Effects of Criteria and Non-Criteria Pollutants from Fossil Fuel Combustion (Theme 1). Unpublished document, Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, December 12.

WORKSHOP COMMENTARY

J. A. Graham: I don't think we're as far apart as some of your statements imply. It seems to me that we all agree that concentration-response studies are necessary; it's just a question of the concentration range over which those studies are to be performed. The straw man document [Miller et al. 1979] uses, I believe, the wording "near ambient levels." We agree that it would be silly to begin a study at an ambient concentration; rather, one should start at a slightly higher concentration and work down. If somebody else had done some research at, say, 0.5 ppm, one might decide to start there.

The straw man offers broad guidelines. When we speak of starting at a "high concentration" and going lower, we would consider 1 ppm to be a "high concentration" for O_3. That is definitely not an ambient concentration; 0.1 ppm would be more likely in an urban environment, although higher concentrations have been reported.

So, in my opinion, we're not so far apart. It's just that our definitions of "high" may be different. Our problem with high-concentration studies also relates to the amount of work involved. In other words, if 100% of the work effort for a given laboratory is at 20 ppm O_3, that represents one category. If 5% of the effort is at 2 or 3 ppm O_3, that's a different case altogether. We're talking about the majority of the research.

It is indeed true that papers on 10 ppm O_3 are not even reviewed for inclusion in the criteria document. The reason is that, with the past O_3 criteria document, there were so many data available in the literature that it became necessary to make a cut somewhere. The flavor we got from all those papers (and for nitrogen oxides toxicology, as well) is that higher concentrations (e.g., 20-30 ppm) might even cause different types of effects. For example, I believe several authors have reported O_3 studies in which high concentrations caused echinosis of the red cells while lower concentrations caused spherocytosis [see Chapter 11 of this volume].

Comment: Those "high concentrations" were 2 to 4 ppm, not 20 to 30.

J. A. Graham: We get in trouble by using words like "high" and "low." The studies showed that 4 ppm caused a different type of effect.

E. L. Alpen: But that's not what you said. I've been peddling my business to the government for nearly 30 years, and I've discovered that when somebody who works for the government writes things down, you're not there to explain them--

J. A. Graham: That's the purpose of this [Research Planning Workshop on Health Effects of Oxidants]. That's why the front page of the straw man uses the word "preliminary."

E. L. Alpen: Toxicology has proven over and over that even very high level studies are extremely useful in providing insights on mechanisms. If one finds high-level studies or dose-dependency curves that go through maxima (and we know lots of these in toxicology), it is crucial to know what side of the maximum one is on.

I would point out one other fact: Almost every major epidemiologic study, whether published or in progress, is not at ambient levels. Such studies focus on high-risk populations, because that's where investigators hope to find something. For example, our group is conducting a hydrocarbon toxicology study for the Department of Energy. We're not out looking at people on the street. Rather, we're looking at three populations of high-risk workers: ramp workers at airports, industrial retail petroleum product deliverers, and petrochemical refinery workers. Most epidemiologic studies are conducted on high-risk populations, because that's where one finds what to look for in the low-risk groups. The same generalization applies in toxicology, too.

J. A. Graham: We in EPA agree.

E. L. Alpen: I don't think we agree.

J. A. Graham: One starts at higher concentrations and works down.

E. L. Alpen: Your position is: If a researcher starts at a high concentration and finds something, he works to lower concentrations, and other investigators should not return to the higher level. My position is: They should, because the first investigator might have missed a lot!

J. A. Graham: It depends on the concentration you're talking about.

E. L. Alpen: I'll just read your own numbers to you: "Any results above 1 ppm O_3 are of no relevance or significance to EPA."

J. A. Graham: The straw man states that they don't go into the criteria document, not that they are "of no relevance or significance."

E. L. Alpen: That doesn't mean they're neither relevant nor significant; they're both, whether they go into the criteria document or not.

J. A. Graham: Here again, we're in terminology.

E. L. Alpen: Would anybody else like to say something?

Comment: As a toxicologist, I understand Dr. Alpen's position very well. But levels above 1 ppm for O_3 and above, say, 5 ppm for NO_2 are so far out of the potential for human exposure under air pollution circumstances that the data from such studies are unlikely to be important to determining end points of biological effects. They may be important to understanding mechanisms, but they're not likely to be important to understanding the potential hazard to a population that is commonly exposed at factors tenfold below this.

With respect to the practical issue of setting standards, I do therefore believe that the limitations recommended in the straw man are appropriate limitations. They are not limitations on toxicology.

J. A. Graham: With respect to your statement on *in vitro* studies, we did not say that they have "no value," period. We said that *in vitro* studies have value under certain circumstances and that those circumstances are quite limited. The reason is that *in vitro* data simply cannot be used for regulatory purposes; they can be used to substantiate an *in vivo* effect, and one might choose an *in vitro* system to help elucidate the mechanism of a certain effect. There are thousands of chemicals to be considered, so one might want to use *in vitro* screening methods. There are other cases where *in vitro* studies are appropriate, but *in vitro* data in and of themselves cannot be used for regulation. So when a decision is made to use *in vitro* exposure experiments, they must be viewed from that standpoint. *In vitro* studies are not of general value; they are of value in certain circumstances, and those circumstances need to be evaluated.

Question: May I have some clarification of the statement, "cannot be used for regulation?" For those of us not familiar with EPA's procedures, is that an internal policy or practice of EPA or is that mandated by some law? Where does the "cannot" come from?

J. A. Graham: Generally speaking, in a criteria document, when a decision is made on what levels are harmful for man and what types of effects occur in man, the emphasis is on the concentration-response data. In other words, one looks at the human data for O_3, let's say, and sees that "X" effects in pulmonary function are demonstrated at a given concentration. An *in vitro* study cannot relate concentration to the human exposure condition as can a human clinical study. When EPA views a human clinical study on O_3, we see what concentrations cause what effects and can then make decisions about safety factors to be included. When one is dealing with an *in vitro* study (no matter what the pollutant), one cannot really use those data in the regulation on a microgram per cubic meter basis.

7. CORRELATING EPIDEMIOLOGIC, CLINICAL, AND BIOLOGICAL RESEARCH ON OXIDANTS

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INTRODUCTION

Developing pollution standards requires us to ask certain basic questions. Typically, the first question is: "How much is too much?" The sequel to that is: "How much are we willing to accept because of economic and other factors?" The first question must be answered by toxicologists, the second question by politicians and philosophers.

Another basic question that is certainly important to those who have the unfortunate and rather unrewarding job of setting standards is: "By which discipline or disciplines do we attack the problem? What can we use to convince people that this agent is toxic at a certain level or not toxic at a certain level?" As a toxicologist, I have always been rather sensitive to that question. My work has generally been with mice; when significant results are obtained, critics sometimes complain: "Well, a mouse is only that long and a human being is this tall. How can they correlate?"

To accumulate data in support of standard-setting activities, three general disciplines are available: epidemiology, clinical experimentation, and biological experimentation. Each carries certain advantages and disadvantages.

Epidemiologic studies are sometimes appropriate for community problems, but epidemiology was originally developed and is more appropriate for relatively simple problems of infectious disease (e.g., when one traces typhoid to a contaminated well). Environmental epidemiologic investigations are plagued with two problems. First, the dose is very low. Secondly, there are innumerable confounding variables. In short, environmental epidemiology carries great potential but is difficult to apply in practice.

Clinical experimentation has the advantage of yielding data that are directly applicable to human beings. There may be difficulties in obtaining dose responses, however, because subjects cannot be exposed to toxicants at concentrations that are much above ambient levels. Also, because of the small number of subjects in any given study, there is a tendency to overlook individual differences in susceptibility or resistance. But such individual differences may be very important, particularly for oxidant air pollutants.

Biological experimentation offers several advantages: higher doses, primary detection of effect, better construction of the dose-response curve, and exposure of relatively large numbers of individuals of defined genetic pattern. Under strictly controlled conditions, it is usually possible to determine whether susceptibility or resistance is the result of genetic structure or is acquired. Of course, the disadvantages are also great. Extrapolation of the obtained experimental data to human beings may be difficult because of discrepancies in lung size, pulmonary frequency, etc. Also, there may be certain biochemical differences between the experimental animals and humans.

It appears, therefore, that the ideal strategy would be to apply these three disciplines in correlative studies that yield unifying answers. The problem is how to do this. How do we detect primary human effect? How do we identify susceptible or resistant subgroups in the human population? How do we develop a dose-response curve? Obviously, dose-response information is best obtained in animals or other biological systems permitting a sufficient spread of dose. If the investigator is limited to ambient or near-ambient levels, the top dose is so small that there is very little room to work.

PREVIOUS RESEARCH

In the case of oxidants, few correlative parameters have been successfully pursued from animal biological through clinical experimental and epidemiologic studies. This is an area deserving more attention.

Eye irritation is a primary problem of oxidant air pollution. This parameter cannot be studied in animals; it is, apparently, a peculiarly human affair. When studied experimentally using artificial smog, eye irritation does not appear to correlate with ozone level or other obvious measurements of oxidant pollution. It is associated, however, with the oxidizing atmosphere.

Pulmonary function is another parameter that has been examined in humans. Pulmonary functions are easily obtained with experimental subjects, and epidemiologic data can be acquired. Also, some information is available on individual differences. For example, in Bates and Hackney's switch experiments, subjects from Canada were found to be more susceptible to oxidant exposure as measured by pulmonary function than subjects living in Los Angeles. Such findings probably reflect some sort of acquired resistance.

A number of parameters measurable in animals may be applicable to human beings. Pulmonary pathology is one of the most sensitive and relatively successful parameters in animals. Oxidant exposure results in definite disease of the small airway. When animals are exposed to appreciable amounts, the resultant lung changes, which include loss of recoil, appear to be rather profound. It appears that no one has attempted comparable measurements in humans; perhaps larger doses would be required. Positive results would suggest that oxidants contribute to pulmonary emphysema in man; already there may be some evidence of this.

Another successful animal parameter is activation of infection. Several studies have shown that animals react to both nitrogen dioxide and ozone at relatively low levels and become more susceptible to bacterial infection of the deep lung, probably as a result of oxidant action on the macrophages and other lung defense systems. Thus, activation of infection would appear to be a highly appropriate model with which to "crosswalk" to humans. With humans there are certain problems, however. First, bacterial pneumonia has become an uncommon disease in man. When it does occur, the disease is frequently secondary to viral infection. Insofar as certain viral infections affect the macrophage system as profoundly as (or more profoundly than) oxidant exposure, human data might be difficult to interpret. Secondly, epidemiologic data for Los Angeles show no correlation between the oxidant season and the "pneumonia season" (if there is such a thing). Perhaps some other area (e.g., Phoenix or Houston) would show a better correlation. Finally, as mentioned earlier, there are problems of species differences and human individual differences.

PROMISING AVENUES FOR FUTURE RESEARCH

One goal for future epidemiologic studies might be to screen subjects to find a susceptible subgroup. Such screening could be based, perhaps, on a correlation between individuals who are exceedingly susceptible to eye irritation and those who demonstrate lung reactivity. Experimental work in Cincinnati showed that some individuals are highly susceptible to eye irritation while others are positively resistant to it. There may be some theoretical objections to using this parameter as a screen: the eye irritation is probably due to other agents within photochemical smog (rather than the oxidant itself). But, as noted earlier, eye irritation does correlate roughly with the oxidizing atmosphere.

Another potentially fruitful line of research would be to investigate whether human "acquired resistance" to oxidants resembles the "tolerance" seen in animals. Over 20 years ago, Stokinger and others showed that low-dose exposures to one gas will protect animals against exposures to large doses and even against exposures to other gases (e.g., phosgene will protect against ozone, or vice versa). In our laboratory, we showed that such results are associated with protection or inhibition of edema. We postulated that this is a laboratory artifact that probably does not occur in nature: the "edemagenic" exposures do not obtain in ambient air, and our cellular studies did not reflect any tolerance phenomenon. Still, clinical experiments have shown that humans do acquire some sort of pulmonary functional resistance to oxidants, and this remains a potentially productive line of inquiry.

One of the obvious places to look for correlations would be in pulmonary function studies. Involved here are noninvasive tests that can be applied across the board in epidemiology, clinical experimentation, and animal experimentation. We already know, of course, that the human response to oxidants entails such physiological responses as constricted airway. The question is: Are these responses significant--are they health-impairing--or do they represent a physiological response that is easily accommodated? The other question is: Does a 4-h acute exposure in animals result in a measurable pulmonary physiological response? In studies performed in the early 1960's, Murphy demonstrated certain changes, including increased frequency of respiration. But he could not convincingly demonstrate any changes in pulmonary resistance, and subsequent studies have been equally unsuccessful. Perhaps the problem is one of primitive techniques: anesthetizing the animals may wipe out these minor changes. To effectively employ pulmonary resistance as a "crosswalk" between animals and humans, then, will likely require us to improve our techniques in animals. There are techniques under development that do not require anesthetization of the animal; this may enhance the response.

CONCLUDING REMARKS

In conclusion, I would suggest that the oxidants problem be reassessed as a "new" problem. Are oxidants a problem? Are the present standards adequate? Rather than reapplying techniques and parameters that have been used in the past, investigators should devote more effort to innovation. We need to develop parameters that provide "crosswalks" from species to species. Only in this way can we develop a coherent body of knowledge with which to approach standard-setting.

8. OVERVIEW OF CURRENT AND PLANNED RESEARCH AT BROOKHAVEN NATIONAL LABORATORY

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LIFE SCIENCES RESEARCH AT BROOKHAVEN NATIONAL LABORATORY

Brookhaven National Laboratory is located approximately 70 miles east of New York City in the geographic center of Long Island. While the major focus at Brookhaven has been in high energy physics, there are a number of departments which conduct life sciences research. The Safety and Environmental Protection Division devotes a portion of its efforts to studying health effects of radiation. The relatively new Department of Energy and Environment (DEE) has several programs associated with health effects research. One group is assessing health effects of population exposures at the national level and of a variety of energy alternatives. A second group in DEE is developing the ability to assess mutagenic and carcinogenic effects of chemicals using short-term in vitro systems. Within the Biology Department, the major focus, of course, is on biological research of all kinds. One effort that relates to oxidants is a program developed by the late Dr. Arnold Sparrow to investigate the mutagenic effects of air pollutants on the higher plant Tradescantia. This study is underway; trailers have been set up within and adjacent to industrial complexes in the Northeast.

BROOKHAVEN INHALATION TOXICOLOGY FACILITY

Inhalation toxicology is a fairly new effort within the Medical Department. Before this author joined Brookhaven in 1976, the only oxidants research being conducted was that of Drs. Schaich and Borg (see Chapter 11 of this volume). The majority of my time at Brookhaven has been spent in developing a laboratory for exposure of rodents to any compound, regardless of the hazard associated with that compound. This facility, which is now operational, is described below.

Facility Layout

The first two figures illustrate the layout of the new Brookhaven Inhalation Toxicology Facility. The inset in Figure 8-1 shows the conceptual layout of the space. On the right-hand side is nonregulated space; on the left-hand side, regulated space. The chambers and glove boxes, indicated by "C" in the inset, are maintained within the regulated space. The air handling system for the nonregulated space is a conventional system with common supply and local exhaust ventilation. The air handling system for the regulated space is completely separate and independent from that of the nonregulated space and has a common exhaust system providing several degrees of filtration for exiting air. A third separate air handling system is provided for the

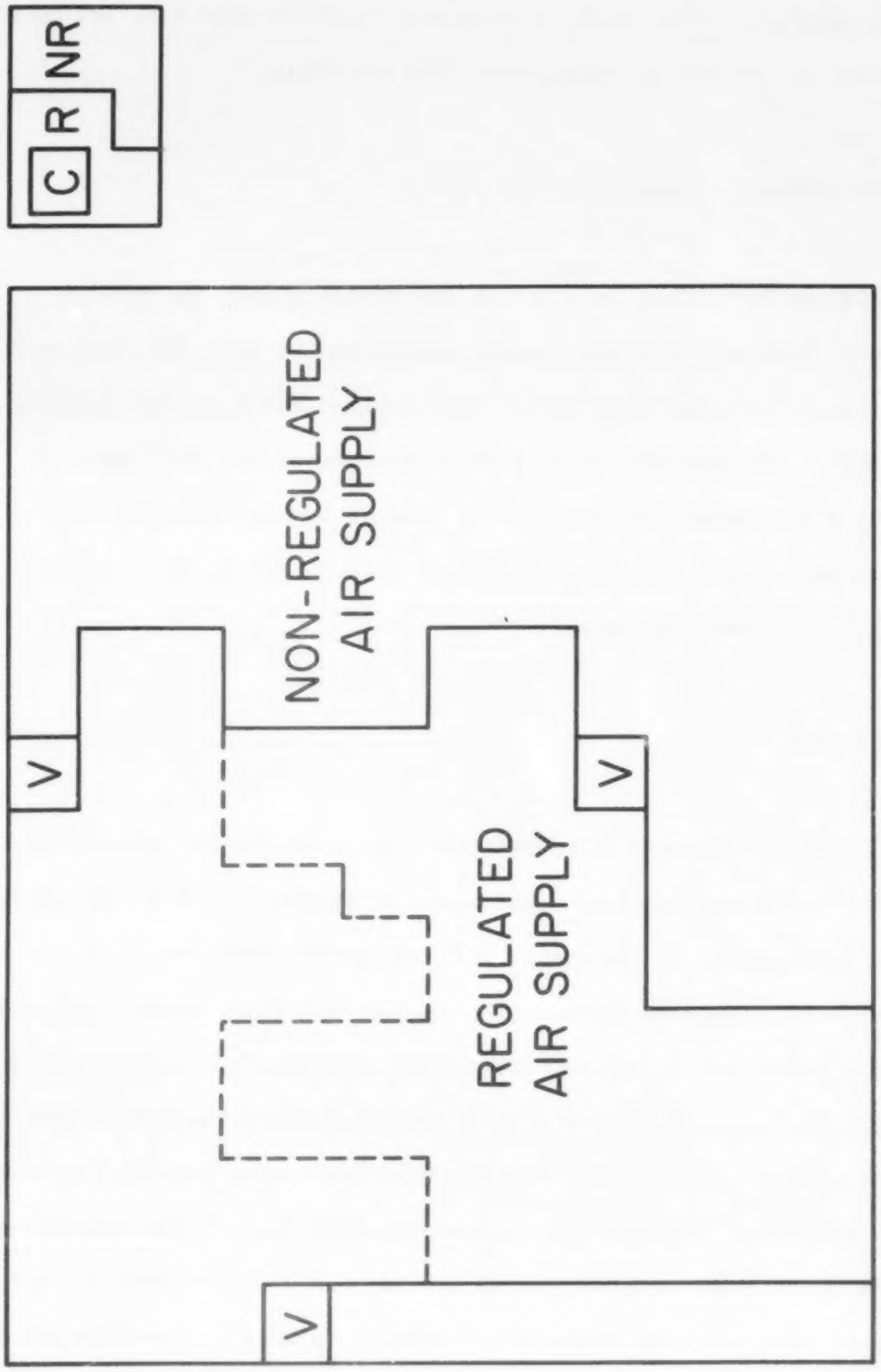
C
R
NR
NON-REGULATED
AIR SUPPLY
REGULATED
AIR SUPPLY
V
V
V

Figure 8-1. Outline of Brookhaven Inhalation Toxicology Facility. Inset: Separation of air handling systems.

inhalation chambers and glove boxes. The air systems for the regulated space and the chambers are totally redundant. In operation, the regulated space is maintained at ~0.08 inH_2O pressure less than the nonregulated space. Chambers are operated at -0.5 to -1.0 inH_2O with respect to the regulated space.

The actual layout of the building is shown in Figure 8-2. Within the nonregulated space are the electron microscopy suite, two conventional laboratories, two offices, and a glass washing area. The regulated space consists of two large chamber rooms separated by a bank of small laboratories; total separation between the two chamber rooms is accomplished by closing one door. We intend to use Chamber Room A for highly hazardous materials and Chamber Room B for more conventional compounds. Two small animal rooms are adjacent to Chamber Room B. Access to the regulated space is through three small vestibules, indicated in Figure 8-1, which are ventilated at ~1 air change/min with clean, fresh air. Two large viewing windows allow observation of operations behind the barrier (Figure 8-3). Thus, visitors can view the exposure facilities without entering the regulated space. Catwalks over the inhalation chambers provide space for contaminant generation and for chamber monitoring (Figure 8-4).

Figure 8-5 is a close-up of one of the 5-ft inhalation chambers. The chambers are installed in pits and arranged so that a rack of animals can be wheeled in. Each rack houses 12 cage units, each capable of holding 8 rats in individual cages. Thus, we can expose up to 96 rats in 1 rack, or a total of 192 rats in 1 inhalation chamber. The cages are capable of housing either 1

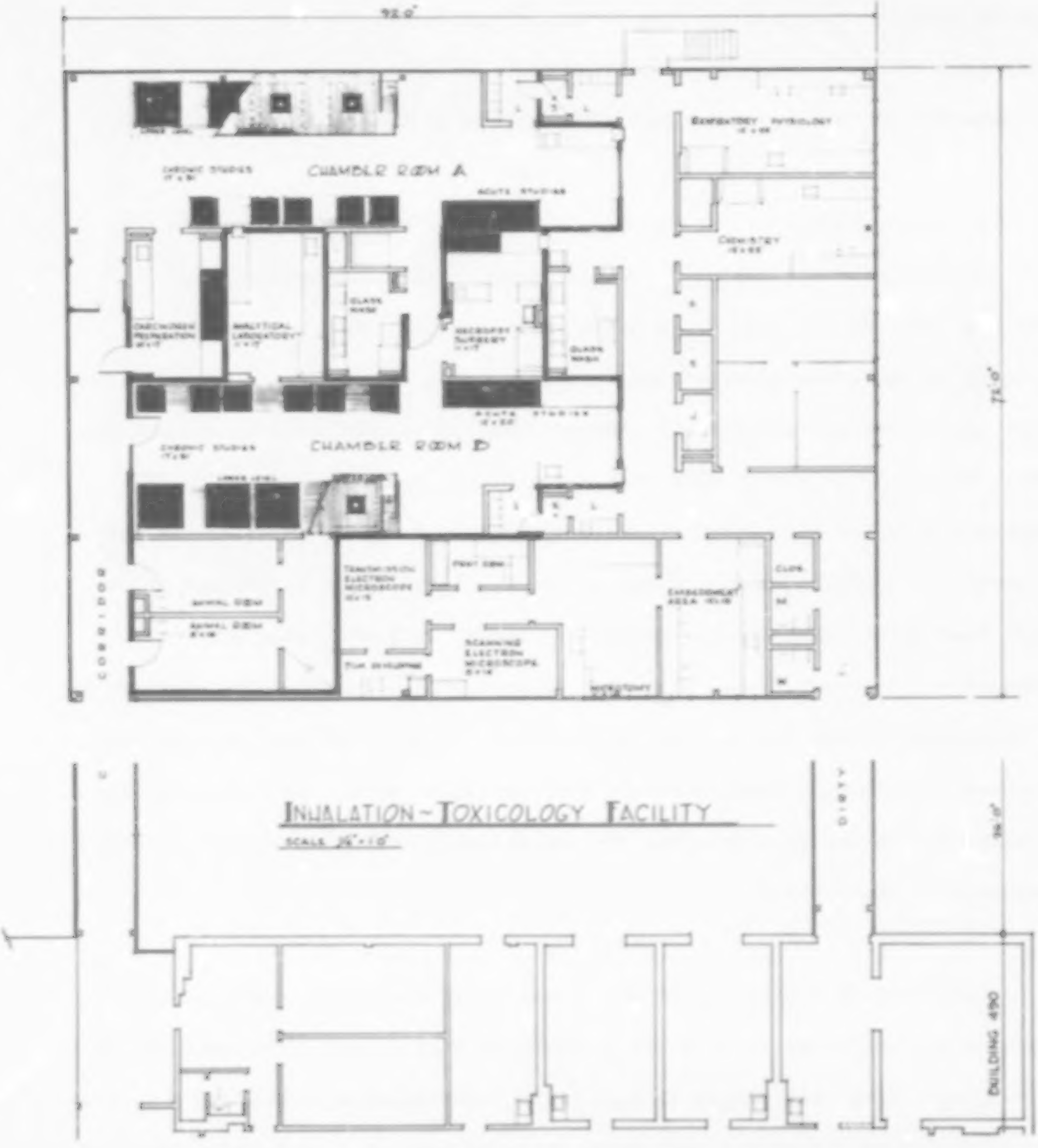
92'0"
CHAMBER ROOM A
CHRONIC STUDIES
ACUTE STUDIES
CARCINOGEN PREPARATION
ANALYTICAL LABORATORY
GLASS WASH
NECROPSY SURGERY
GLASS WASH
ACUTE STUDIES
CHRONIC STUDIES
CHAMBER ROOM B
UPPER LEVEL
RESPIRATORY PHYSIOLOGY
CHEMISTRY
74'0"
CORRIDOR
ANIMAL ROOM
ANIMAL ROOM
TRANSMISSION ELECTRON MICROSCOPE
PRINT ROOM
FILM DEVELOPING
SCANNING ELECTRON MICROSCOPE
MICROTOMY
EXPERIMENTAL AREA
CLOS.
INHALATION-TOXICOLOGY FACILITY
SCALE 3/16"=1'0"
DIRTY
BUILDING 490

Figure 8-2. Floor plan of Brookhaven Inhalation Toxicology Facility.

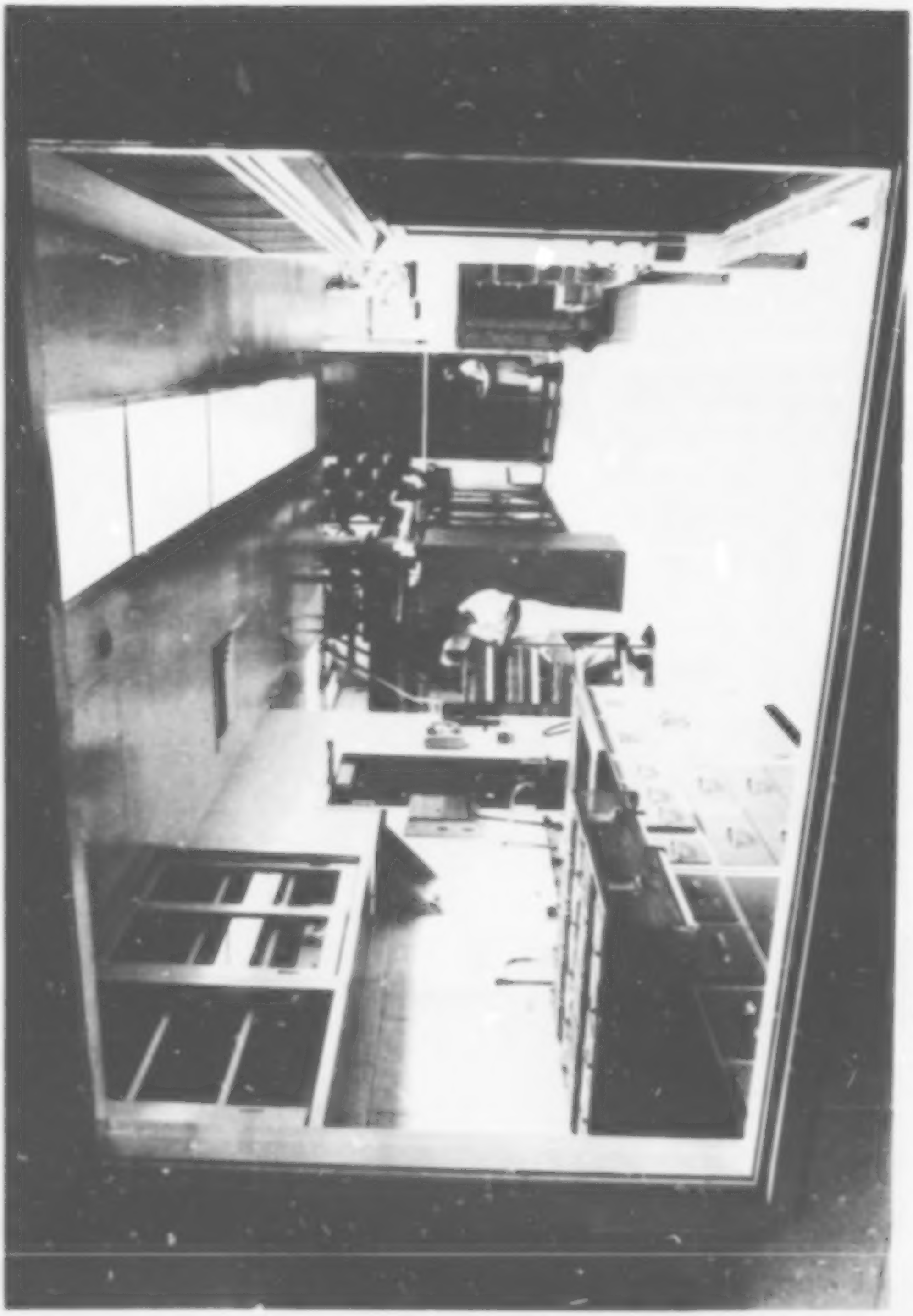

Figure 8-3. Chamber Room B (through the viewing window).

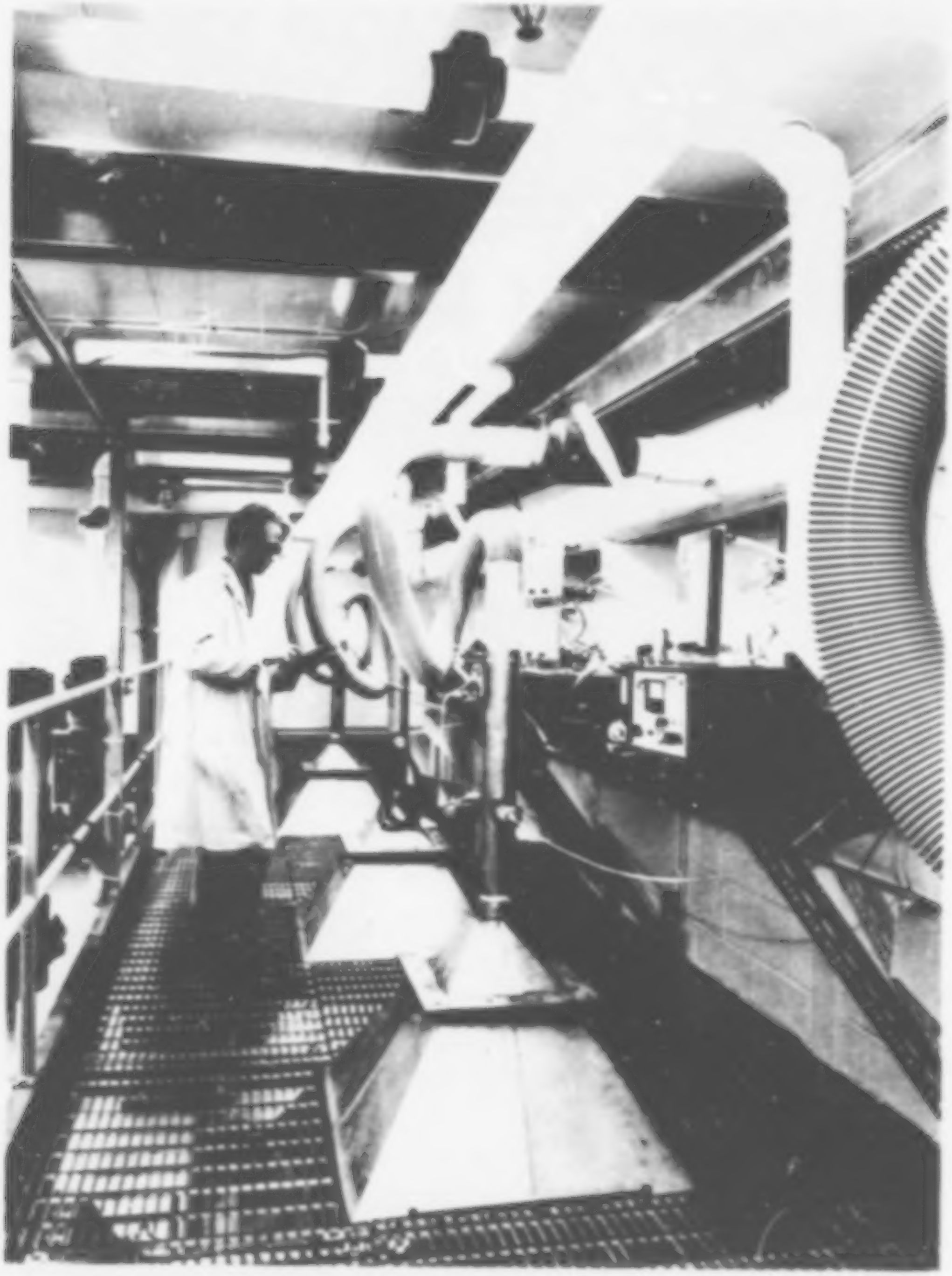

Figure 8-4. Generation and monitoring equipment located above the chambers.

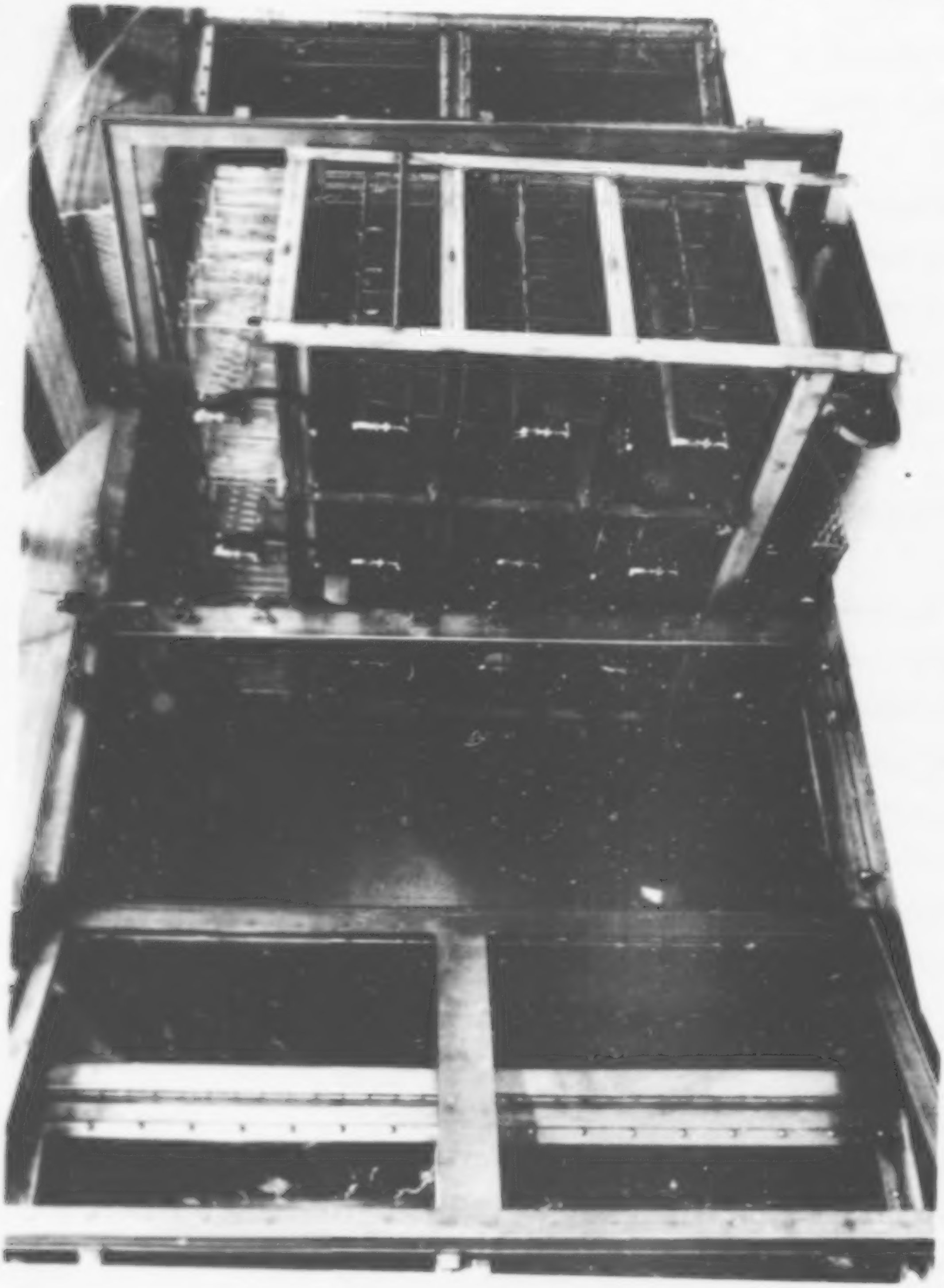

Figure 8-5. Close-up of caging unit.

rat, 2 hamsters, or 3 mice; thus, this design is capable of exposing up to 576 mice in 1 system.

These facilities came on-line in December 1979 and are now in operation. Chamber Room A will be used to expose animals to highly hazardous compounds, such as polycyclic aromatic hydrocarbons and other known carcinogens. For these compounds, we intend to employ chambers that will house and expose animals in a totally attached unit. Design of these chambers is underway.

Instrumentation and Techniques

Our goal is developing this research effort was to build a team capable of measuring functional or physiological changes, anatomical changes, and biochemical changes in small animals. To that end, we recruited a small animal respiratory physiologist, Dr. Daniel Costa, who trained under Dr. Mary Amdur. He has purchased and assembled equipment necessary for measuring the physiological status of the rat pulmonary system in terms of spirometry and mechanical function. We are using plethysmography and gas dilution techniques We believe these methods represent the state of the art.

We will employ a recently acquired scanning electron microscope to assess the anatomical structure of lungs of animals exposed to a variety of airborne materials. Conventional pathologic evaluation will be included, and we intend to develop morphometric techniques at the level of the light microscope. In addition to histopathologic evaluation, we will also measure a number of lung biochemical indices which indicate response to challenges of environmental agents.

Current and Planned Studies

Projects that are underway include: a study of the deposition and translocation of sized glass fibers, supported in part by the Thermal Insulation Manufacturers Association; a comparison of functional change versus anatomical change after exposure of rodents to a series of known pulmonary toxicants (ozone was one of the first compounds chosen as a model pulmonary toxicant); a study of the interaction between hypertension and air pollution (see Chapter 10 of this volume); the use of bleomycin to produce a model of fibrosis in the rat; and exposure of animals to coal dust in order to develop a rodent model of coal workers' pneumoconiosis.

Since epidemiologic evidence shows that people who are already sick are at a much greater risk during episodes of high air pollution, we intend to develop animal models of various aspects of chronic lung disease. Having developed these models, we hope to then superimpose exposure to some of the common air pollutants (such as nitrogen dioxide and ozone) in an attempt to understand why already-stressed persons are at a greater risk during episodes of high air pollution.

9. INCORPORATING OXIDANTS IN ASSESSMENTS OF ENERGY-RELATED HEALTH EFFECTS

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THE NATURE OF A HEALTH EFFECTS ASSESSMENT

A health effects assessment is a documented, quantitative description of knowledge and uncertainty regarding the potential health impact of a program or policy action. Such assessments are useful in evaluating or formulating research and development in both the energy and environmental areas.

An assessment considers the potential health implications of an installed future energy industry, for example, based on current knowledge of the technology, its environmental residuals, and effects on workers and the general population. Computer modeling and simulation studies form the framework of such an evaluation. Figure 9-1 is the schematic outline of a model for air pollution effects. One or more scenarios are developed, plants are sized and sited, and other activities (e.g., automobile traffic) are distributed geographically. Based on technological characterizations, emission estimates are made. Air transport, dispersion, and chemical modeling

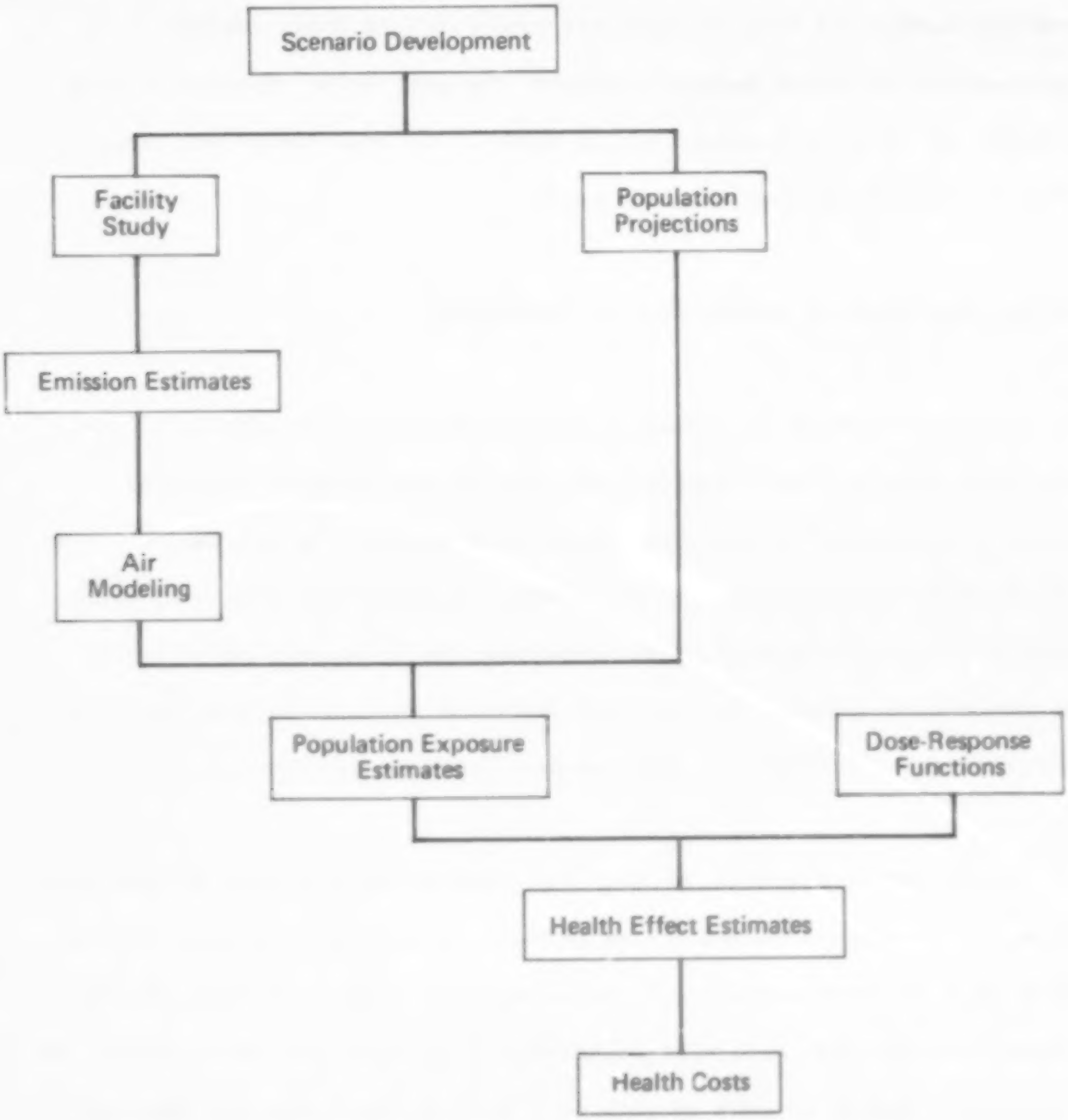
Scenario Development
Facility Study
Population Projections
Emission Estimates
Air Modeling
Population Exposure Estimates
Dose-Response Functions
Health Effect Estimates
Health Costs

Figure 9-1. Model for assessing the health effects of energy-related air pollution.

are performed. The results of these activities are combined with population projections to estimate population exposure. Such factors as population time-activity patterns are often included. In some cases, detailed lung and metabolic models are used to translate exposure into dose. Using dose-response or health damage functions, the population exposure or dose estimates are used to estimate health damage. In some instances, health damage is translated into monetary terms.

SPECIAL CHALLENGES OF ENERGY-RELATED ASSESSMENTS

One major problem in assessing energy-related health effects is our near-total lack of health damage functions for the kinds of low-level exposures expected from most new energy developments. We are dealing in an area of great uncertainty. The uncertainty is compounded when we project emission characteristics of a new technology for which only pilot plant measurements may exist. In fact, the degree of uncertainty may itself be a more important consideration than our best estimate of effect.

To the extent possible, we must deal explicitly with this uncertainty. One way is to express parameters as probability density functions (pdf's) rather than as "best estimates." As an example, Figure 9-2 displays the probability that one particular coefficient will fall in a given range. It takes some judgment to come up with such density functions, but they are important when one needs to combine several uncertain independent parameters. It would be very unlikely that all would be at the high (or low) end of the

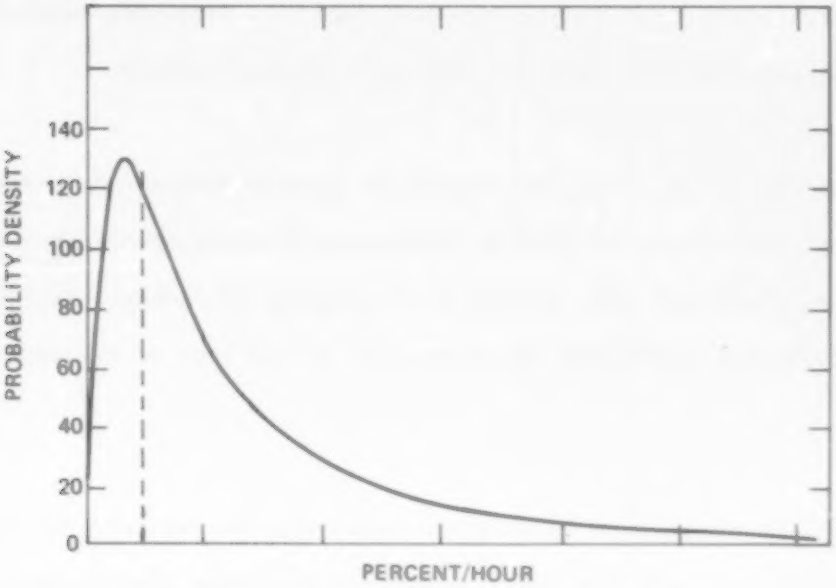

Figure 9-2. A probability density function.

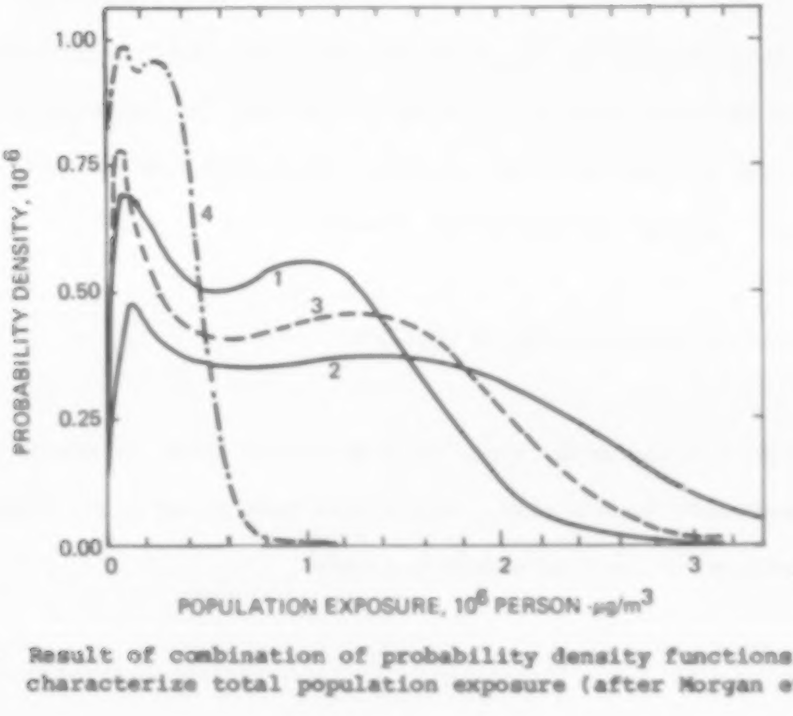

Figure 9-3. Result of combination of probability density functions to characterize total population exposure (after Morgan et al. 1978).

range at once. Figure 9-3 illustrates the result of combining several pdf's to yield the likelihood of various population exposure levels.

Another way to use assessment models is through sensitivity analysis, where we test the effects of various hypotheses of environmental transport, dose-response functions, etc. on the final estimate of damage. This can be particularly useful in finding the importance of one line of research over another.

Ideally, we would prefer a complete dose-response function for man in the range of exposure. Certainly this is what investigators should aim for. Realistically, we settle for much less, and it becomes important to draw information from many sources to form a hypothesis of the function. High-dose information helps define the shape of the curve and identify mechanisms. *In vitro* studies may also provide clues to mechanisms. In addition to helping guide the design of ambient level studies, these data lead to hypotheses that can be directly applied in assessment models.

INCORPORATING THE CONSIDERATION OF OXIDANTS

With regard to oxidants, what has been accomplished in energy-related health assessments? Very little. There have been a number of assessments of the health effects of various energy systems:

An Assessment of National Consequences of Increased Coal Utilization (U.S. Department of Energy 1979a)

Regional Issue Identification and Assessment (U.S. Department of Energy 1979b)

Energy in Transition, 1985-2010 (National Research Council 1980)

The Environmental Impacts of Production and Use of Energy (Part I, Fossil Fuels) (United Nations Environment Programme 1979)

None of these includes quantitative assessments of the health effects of oxidants. Estimates have generally been based on sulfur and particulates; this is a major shortcoming. For example, in the first study listed above, increasing emission controls were projected to lead to decreasing sulfur dioxide and sulfate levels despite a considerable increase in coal consumption. Nitrogen oxide emissions and presumably ozone levels, however, would increase. These were not included for two reasons: First, a credible atmospheric model having a large regional basis is not available. Thus, it is impossible to make estimates of population exposure. Second, no acceptable health damage function is available. The first problem is expected to be resolved by 1982; the latter is of more interest here.

There have been several difficulties in developing an oxidant health damage function. Epidemiologic studies are generally negative or inconclusive. Clinical and animal studies show strong effects, but these effects generally involve subclinical physiological factors. Changes in lung compliance, pulmonary function, and blood chemistry are difficult to relate directly to morbidity and mortality. We have designated substantive disease or death as the only end points qualifying for consideration. This stems, in part, from the fact that the health effects documented in our assessments are in some sense "weighed" (by others) against the cost of controls. We do not feel likely to convince anyone to enter into a multi billion dollar control program on the basis of predictions of subclinical effects. Even large numbers of headaches and respiratory symptoms do not constitute a strong counterweight.

RESEARCH NEEDS

What can be done? First, we need more information on the contributions of oxidants to chronic disease. As we all know, there are some suggestions of chronic respiratory disease. One way to attack this problem is through animal models of chronic disease. Another avenue is further epidemiology. Still another possibility is the indirect route of linking changes in pulmonary function, changes in blood chemistry, biochemical effects, and minor respiratory symptoms with chronic disease.

Secondly, we can form hypotheses on the relation of oxidants to chronic respiratory disease and test the implications of those hypotheses in assessment models. Sensitivity testing can provide some notion of the degree to which changes in the hypotheses affect predictions of overall effect. It appears that we will have to build models that are much more specific about sensitive subpopulations, physical activity, and (perhaps) change of exposure level with time.

ACKNOWLEDGMENT

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10. INTERACTIONS BETWEEN HYPERTENSION AND OXIDANT AIR POLLUTANTS

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INTRODUCTION

In the 1960's when the word "relevance" was not so important, one very interesting project at Brookhaven National Laboratory was an investigation of the causes of hypertension. By developing an animal model of hypertension, the late Dr. Lewis K. Dahl proved that both genetic and environmental factors are involved in the development of hypertension. Over the ensuing 15 years or so at Brookhaven, we have refined this model, selectively breeding two lines of rats from the same initial breeding stock. One of the lines is susceptible (S) to salt-induced hypertension, and the other is resistant (R) to salt-induced hypertension (Figure 10-1). We have used this model to investigate interactions between commonly encountered air pollutants and the development of hypertension. Since the experiment is loaded in favor of producing a positive effect, a negative finding (i.e., one in which no increased effects occur subsequent to a significant challenge by the pollutant in question) can be interpreted favorably. This paper summarizes our studies investigating the interaction of two air pollutants, sulfur dioxide (SO_2) and ozone (O_3), with the hypertensive model.

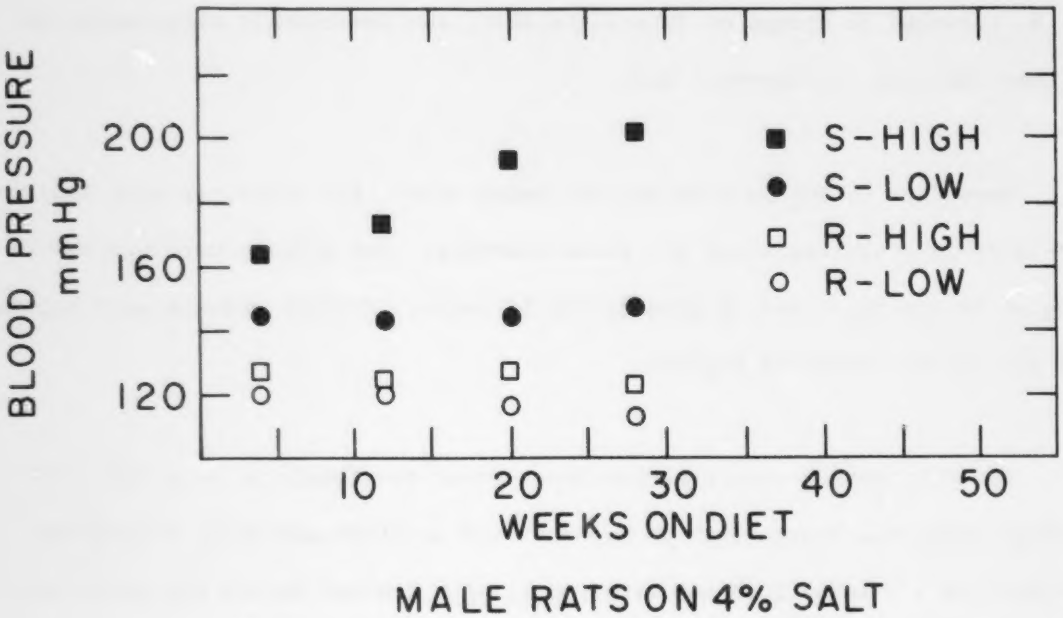

Figure 10-1. Blood pressure of S and R male rats on high- and low-salt diets as a function of time.

METHODS

Both studies were designed as 2 x 2 x 2 matrix studies with 3 variables: air vs. pollutant, high dietary salt vs. low dietary salt, and S vs. R line of rats. This design required 8 groups of 10 rats each.

Sulfur Dioxide Study

For studies with SO_2, the high-salt diet consisted of 4% salt; the low-salt diet, 0.4% salt. Forty male S rats and 40 male R rats were weaned at 21 d, arranged in groups of 10 animals each, and immediately established on either the high- or low-salt diet.

Exposures to SO_2 started the following week. All exposures were done in 27- x 27-in stainless steel and glass chambers. The animals were exposed to SO_2 at 50 ppm for 6 h/d, 5 d/week, for 31 weeks. Control animals were exposed to air for the same 6-h regimen.

Blood pressures were measured under ether anesthesia using a tail cuff method after the fourth week of exposure and on alternate weeks thereafter. Animals were allowed food and water ad libitum (except during exposure) and were housed under a 12-h on/off light cycle. The measurements were made immediately after exposure to SO_2 on Thursday and Friday.

Ozone Study

Ozone exposures were carried out in similar chambers at a concentration of 2 ppm for 6 h/d, 5 d/week, for 20 weeks. In this study, however, female rather than male rats were used. The animals were allowed 1 week from the time of weaning until establishment on the low- or high-salt diet. The high-salt diet was 8% salt (rather than 4% salt as in the SO_2 experiment). In addition, because of the limited availability of sibling animals, each group was divided into subgroups of 5 animals. Thus, 20 S females and 20 R females were weaned on 2 consecutive weeks prior to random assignment to 1 of the 8 experimental groups. Exposures of the first sibling group (half of the animals) began 1 week prior to exposures of the second group. Subsequently, all animals were exposed simultaneously. Blood pressure was measured after the fourth week of exposure and on alternate weeks thereafter. Data were accumulated on the basis of exposure duration.

RESULTS AND DISCUSSION

Sulfur Dioxide Study

Figures 10-2 and 10-3 show blood pressure as a function of time for SO_2 animals on low and high dietary salt, respectively. The figures clearly show that the initial blood pressures in S animals were markedly higher than in R animals. It is also evident (Figure 10-2) that there were minimal differences over the course of the experiment between SO_2- and air-exposed rats fed low-salt diets. The mean blood pressure of S animals maintained on high-salt diets increased as expected (Figure 10-3). In this case, SO_2-exposed animals had blood pressures higher than those of air-exposed counterparts. This difference was statistically significant at certain time points while not significant at others. However, all differences disappeared after the last exposure to SO_2.

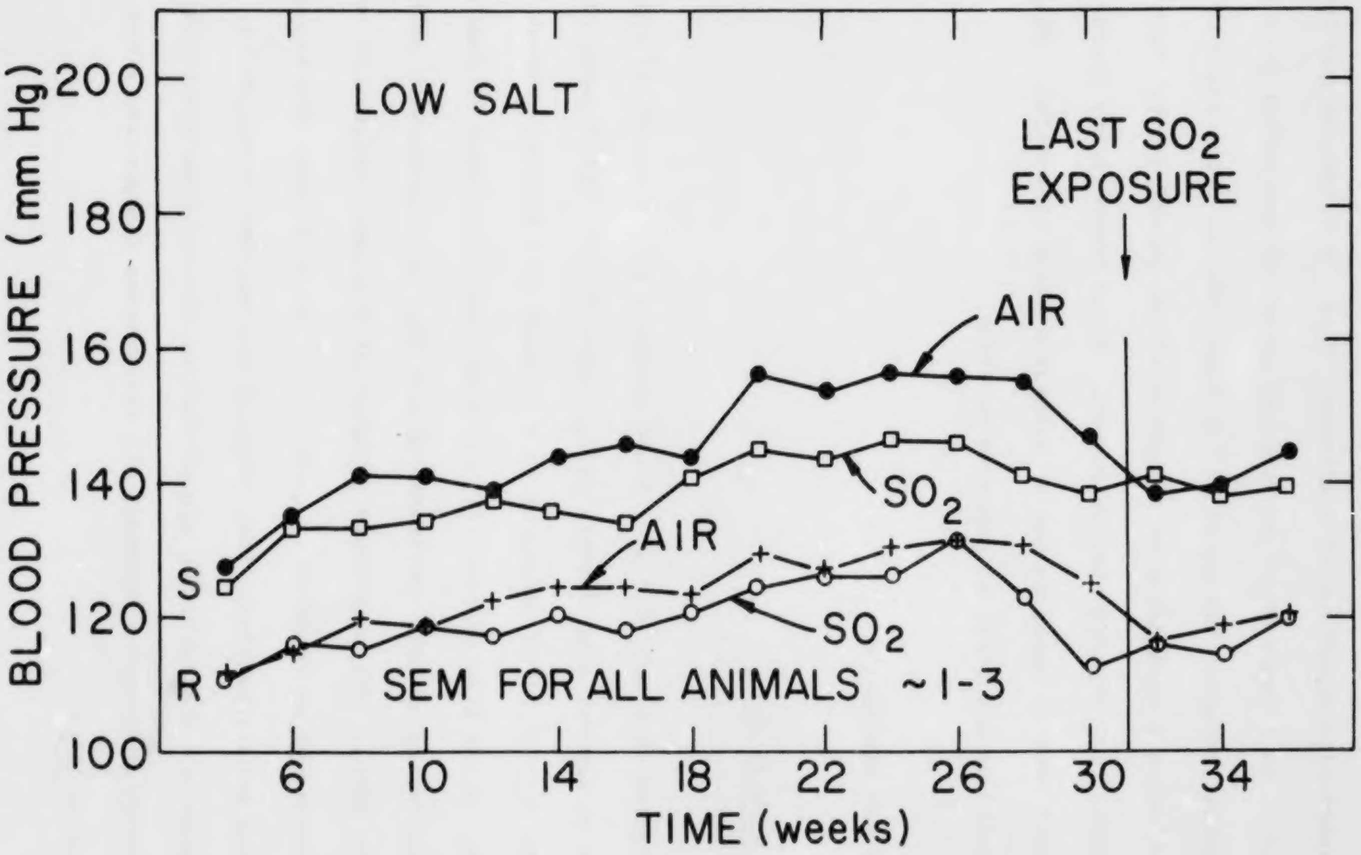

Figure 10-2. Blood pressure of S and R male rats exposed to air or SO_2 and maintained on low dietary salt.

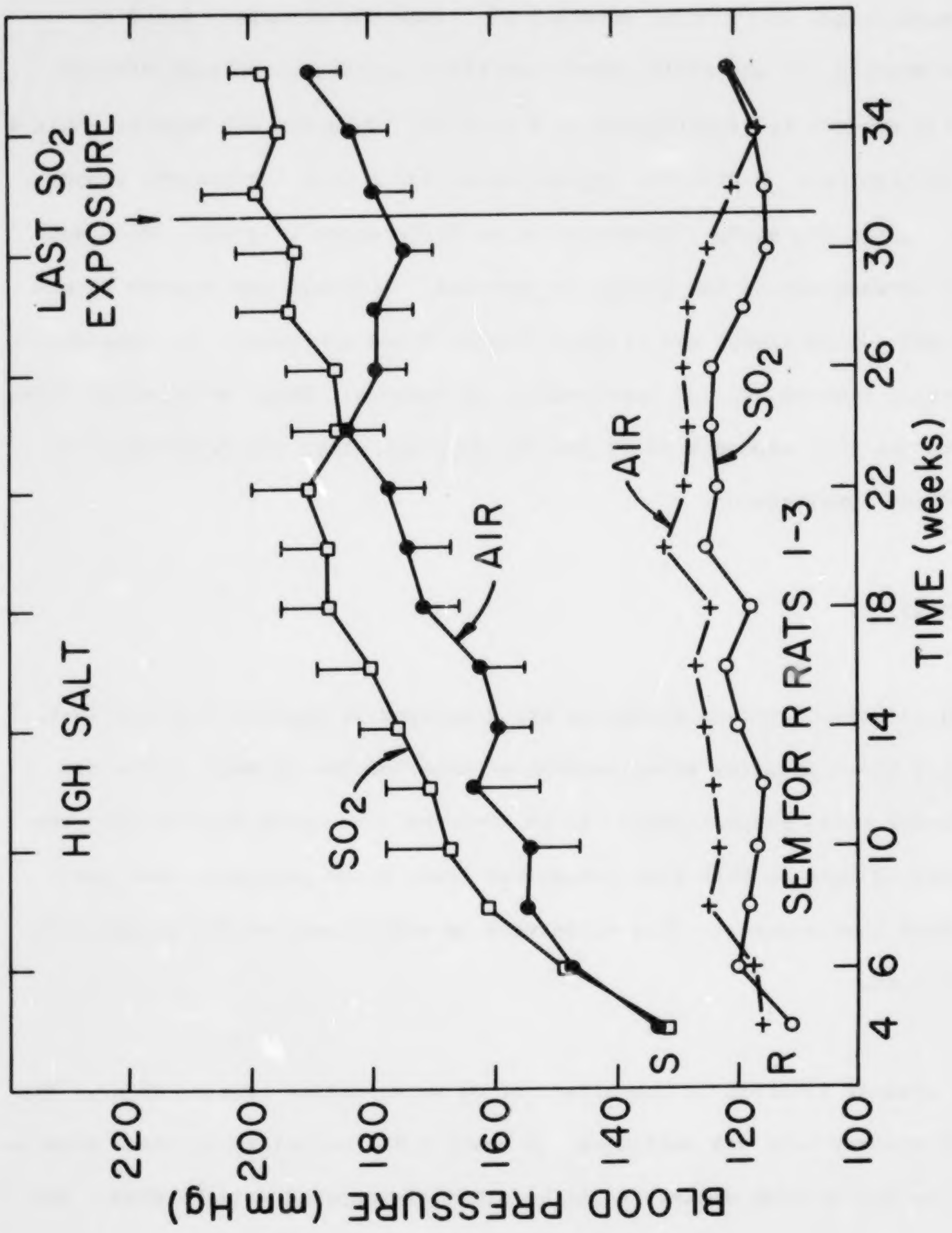

Figure 10-3. Blood pressure of S and R male rats exposed to air or SO_2 and maintained on high dietary salt.

In these studies, the percentages of dietary salt and the concentration of SO_2 were chosen in order to severely challenge the animals. Based on previous studies, it was anticipated that the S animals given high dietary salt would succumb to hypertension in 4 to 6 mo. This did not happen. Only 4 S rats on high salt, 2 from the exposed group and 2 from the control group, died during the SO_2 study. Furthermore, no differences in growth rates were observed between any of the groups in question. Although the exposed S rats on high-salt diets always had slightly higher blood pressures, any apparent relationship between SO_2 and hypertension is tenuous. Thus, we conclude from these studies that exposure to 50 ppm SO_2 does not alter the development of salt-induced hypertension.

Ozone Study

The results of our O_3 exposures are displayed in Figures 10-4 and 10-5, which plot blood pressure as a function of time for the animals on low and high dietary salt, respectively. It is apparent from these figures that the O_3-exposed animals in both diet groups had lower blood pressures than their air-exposed counterparts. This difference is significant at the probability level of 0.05.

Of greater interest is the effect of O_3 on mortality (Figure 10-6). The level of dietary salt was sufficient to cause a 50% mortality in the S animals exposed to air on high dietary salt; no other air-exposed animals died. The O_3 challenge (2 ppm for 6 h/d, 5 d/week) was sufficient to cause 3 and 2

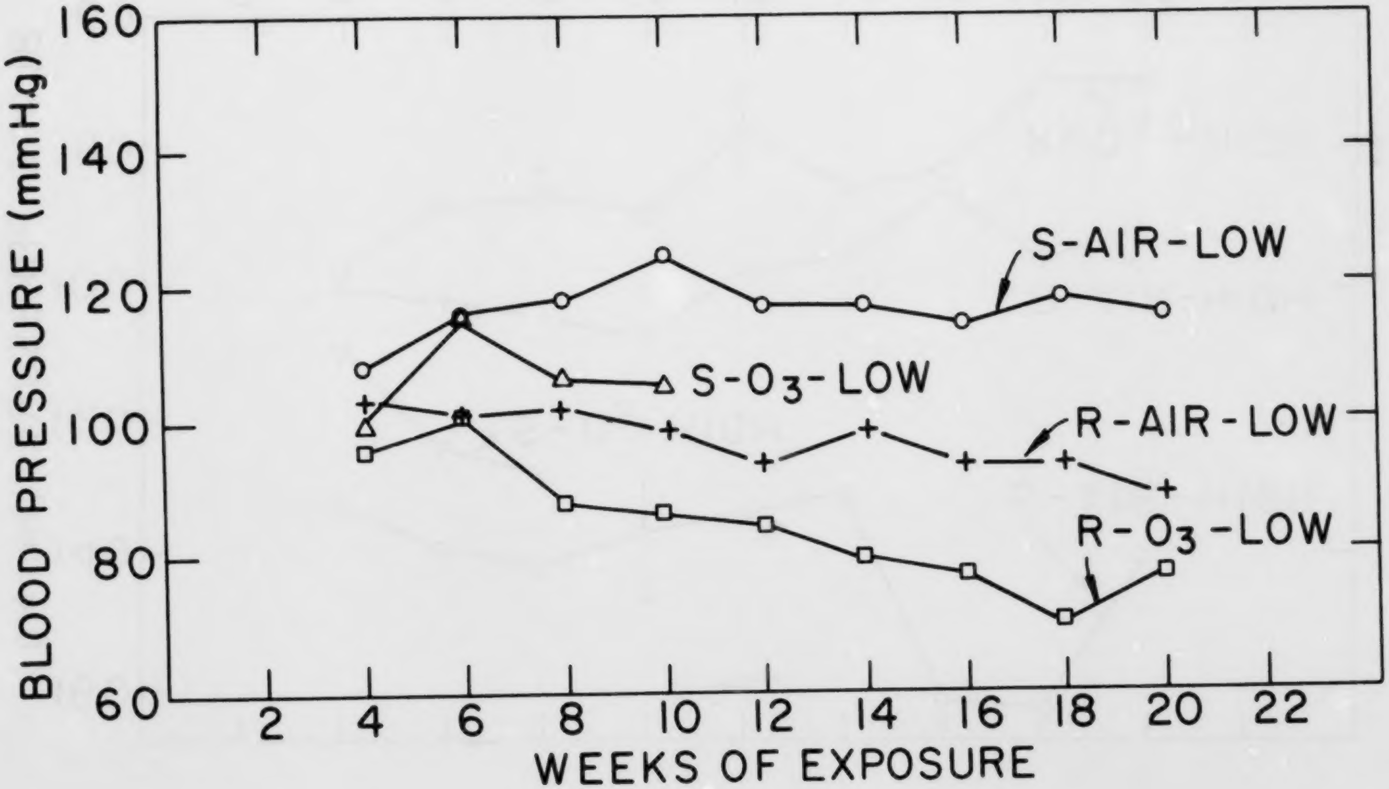

Figure 10-4. Blood pressure of S and R female rats exposed to air or O_3 and maintained on low dietary salt.

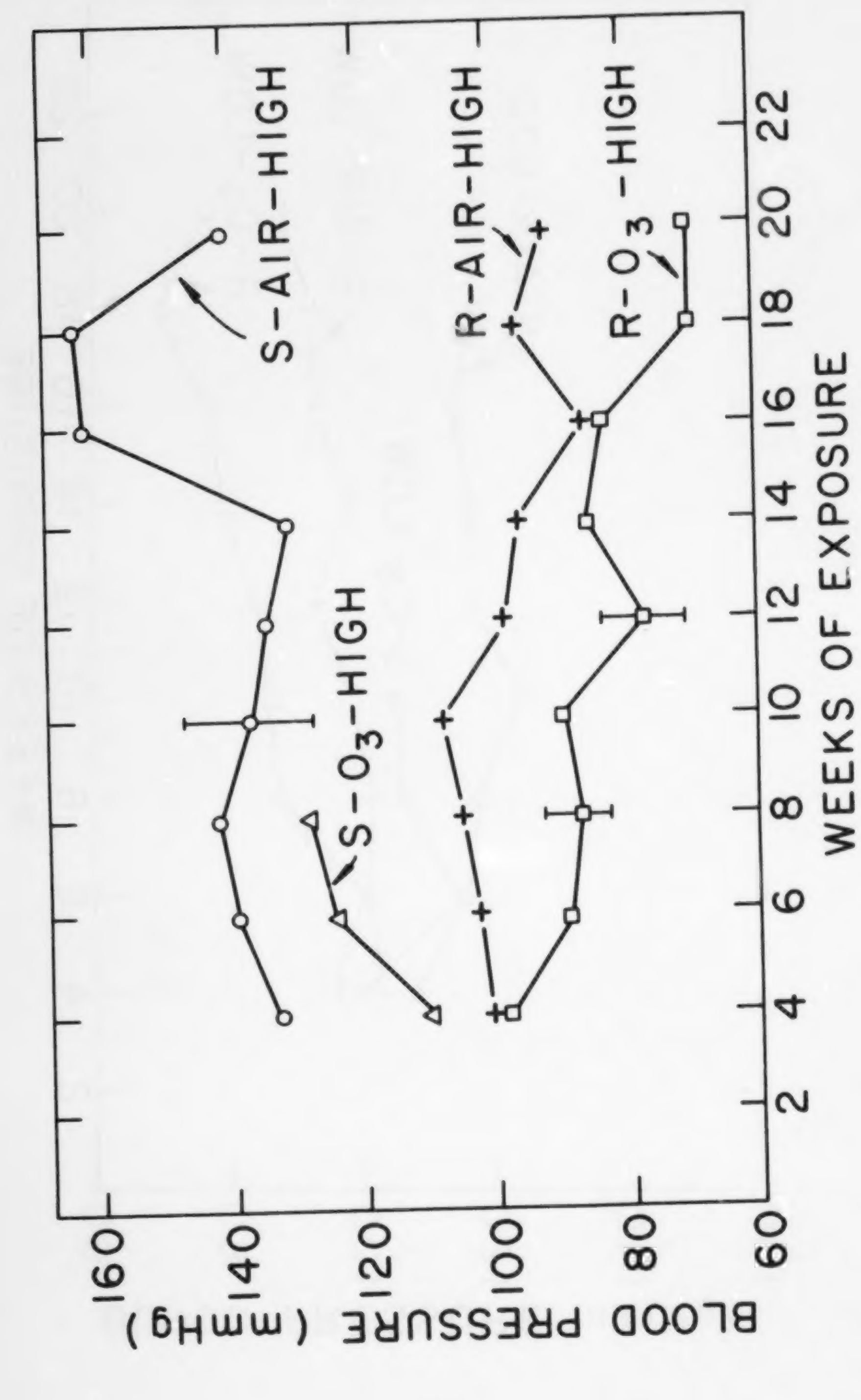

Figure 10-5. Blood pressure of S and R female rats exposed to air or O_3 and maintained on high dietary salt.

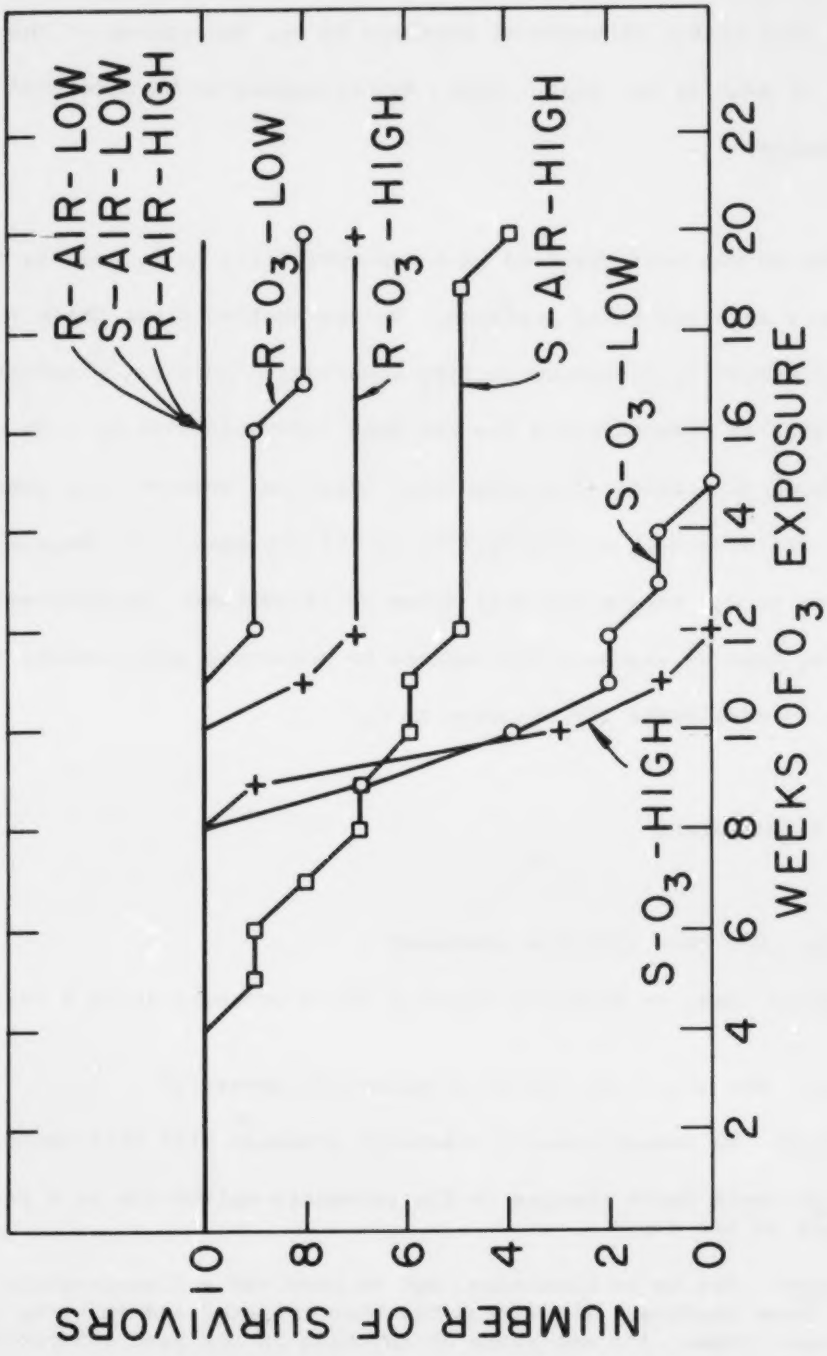

Figure 10-6. Mortality of S and R female rats exposed to air or O_3 and maintained on high or low dietary salt.

deaths in the R rats on high or low salt, respectively. In contrast, all S animals died within 16 weeks of exposure to O_3, regardless of the presence or absence of salt in the diet. These deaths appear to be unrelated to hypertension.

What we may have observed is a susceptibility to O_3 that is independent of dietary salt and blood pressure. We are excited about these results, since this difference in O_3 susceptibility with regard to S vs. R animals may be very useful in understanding how the lung interacts with O_3 from a biochemical/physiological standpoint. There is, however, the possibility of a general difference in susceptibility to all stresses. If the susceptibility is unique to O_3, this model will allow us to evaluate the biochemical changes resulting from O_3 exposure and perhaps to intervene with various treatment regimes to ameliorate the response to O_3.

WORKSHOP COMMENTARY

Question: Was this systolic pressure?

R. T. Drew: Yes, we measured systolic blood pressure using a tail cuff method.

Question: Was there any change in diastolic pressure?

R. T. Drew: We cannot measure diastolic pressure with this technique.

Question: Were these changes in the pulmonary epithelium as a result of the high salt in the diet?

R. T. Drew: Not to my knowledge, but we have not yet systematically evaluated all of these studies. The tissue has been prepared and is being evaluated at the present time. I'm not aware of anything in the past literature or in any of Dahl's previous work that suggests that there would be differences in the respiratory epithelium.

J. D. Hackney: Is it possible that these animals are susceptible to any kind of stress?

R. T. Drew: Yes, it is possible. This is one of the reasons that I'm a little reserved when I say that we may have a biochemical model; there is a suggestion that S rats are generally susceptible to stress. I don't know exactly what this means.

Question: Did O_3-exposed animals lose more weight?

R. T. Drew: In animals exposed to SO_2, there were no differences. The week that the exposure started, the SO_2-exposed group did not gain weight. From then on, all groups gained at comparable rates. The SO_2-exposed group never caught up and was always a little bit lighter. With O_3, there were some differences at the start of the study. This is unfortunate, but when you're working with a limited number of animals you really can't select for uniform weight as with a larger number of animals. However, in every case, O_3-exposed animals weighed less than their air-exposed counterparts.

Question: Did you identify the factor causing hypertension?

R. T. Drew: We believe it is a genetic factor.

Question: Is it a kidney or a vascular problem? What's causing the animals to be more susceptible to high salt?

K. M. Schaich: There have been very few studies to characterize these rats in terms of physiology and biochemistry. There were some early studies in which hormonal effects were sought, but none were observed that could cause the increased blood pressure. Presently, we are working on biochemical characterization of these rats, but we don't yet know how they differ. There is some evidence that liver function may be different in the S vs. R rats, but we don't have detailed information yet.

Question: Is there any difference in the pathology of these animals? Do they have more extensive lesions? Do the lesions appear earlier? Obviously at 2 ppm the animals will develop lesions.

R. T. Drew: Dr. Slatkin, a colleague, is working on this evaluation now. I can't answer the question. The animals that died in the O_3 study clearly died a respiratory death. It was not a hypertensive death; on gross pathology, the lungs were deeply involved (as one would expect). The micropathology is yet to come.

We've done respiratory function studies on the R animals in this group (there are no S animals left). Our results appear consistent with small airway disease.

Comment: With that concentration, you might be getting some effect on the converting enzyme. Have you looked at that?

R. T. Drew: No, we haven't. This is an exciting observation and we will certainly pursue it.

Comment: I don't know the data on ordinary species of rats. Is it possible that you have a very resistant strain as opposed to a sensitive one? Was the difference because one strain is extraordinarily resistant to this very high level of O_3?

R. T. Drew: I don't believe so. I don't believe my data on O_3 toxicity are in conflict with the literature on mortality alone.

Comment: That's what I'm asking. What is the experience with other strains?

R. T. Drew: Well, I'm presently exposing Fisher 344 rats to O_3 for the National Toxicology Program. We are exposing animals to 0, 0.2, 0.8, and 2 ppm O_3 and are beginning to see deaths in the 2 ppm group 8-9 weeks after beginning the exposures. So, the results are consistent.

Question: Are you saying that your S rat is more susceptible to the same doses of O_3 than other strains of rats?

R. T. Drew: Yes, I think that is correct.

11. THE EFFECTS OF OZONE ON RAT ERYTHROCYTES AFTER EXPOSURE IN VIVO

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INTRODUCTION

For some time, concern has been expressed that toxic effects of ozone (O_3) may not be limited to the lung but may extend to other body tissues. Most biochemical investigations of systemic toxicity have focused on erythrocytes: the cells which have first contact with the exposed lung and which, along with plasma, presumably will be the carriers of cytotoxic potential to other sites. Observations from a large number of studies in several species have been published (Table 11-1), but the issue of extrapulmonary effects of O_3 reflected in red blood cells is anything but clear. As can be seen in Table 11-1, a variety of end points have been monitored on blood, including hematological/morphological, physiological, and biochemical/metabolic. There has been little effort, however, to integrate different types of effects into an overall pattern of response. In terms of cellular biochemistry, most statistically significant changes have been noted only when rats were made tocopherol (vitamin E) deficient before exposure or

TABLE 11-1. EFFECTS IN RED BLOOD CELLS FOLLOWING IN VIVO EXPOSURE TO OZONE

Species	Exposure Conditions	Observations[a]	Reference
rat	1.5 ppm x 3 d 6 ppm x 4 h 8 ppm x 4 h	no effect SOD, GPx, K^+ flux ↑retics. (all levels) ↑Hb, Hct, echinocytes II & III (6 & 8 ppm) (echinocytes correlated with petechia in lungs, indicative of vascular endothelial damage)	Larkin et al. 1978
rat Sprague-Dawley male 3 mo ± 45 ppm tocopherol	0.8 ppm continuous x 7 d	-Vit. E: (↓)GSH, ↓GPx, ↑PK $+O_3$: ↓GSH, ↑GPx, ↑PK ↑LDH, no effect SOD, catalase, TBA, G6PD MetHb, reticulocytes +(Vit. E + O_3): no O_3 effects	Chow and Kaneko 1979
mouse Caesarian-delivered-1 male adult	0.85 ppm x 4 h	↑Heinz bodies (↓with continued exposure)	Menzel et al. 1975
mouse	8 ppm x 4 h	↓AChE, (↓)GSH, ↑Hb	Goldstein et al. 1968
rat Sprague-Dawley male	5 ppm x 1.5 h	↑H_2O_2, ~50% ↓catalase	Goldstein 1973
mouse	6.7 ppm x 1.5 h 1.7 ppm x 1.5 h 1.0 ppm x 4 h	↑H_2O_2, ~16% ↓catalase no effects no effects	Goldstein 1973
rat Sprague-Dawley male 5 mo ± 45 ppm tocopherol	0.8 ppm x 7 d	$+O_3$: ↑GPx, ↓GSH (both groups) -Vit. E: ↓GPx, GSH ↑G6PD & 2,3-DPG	Chow et al. 1976
rat Sprague-Dawley male 2 mo	0.5 ppm x 8 h/d x 7 d	lung: ↑GSH, GPx, GRase, G6PD (20-25%) RBC's: nonsignificant ↑GPx, ↓GSH	Chow et al. 1975

(continued)

TABLE 11-1 (continued)

Species	Exposure Conditions	Observations[a]	Reference
rhesus monkey male 2 yr	0.5 ppm x 8 h/d x 7 d	lung: ↑GSH, GPx, GRase, G6PD (10-15%) RBC's: nonsignificant ↑GPx, ↓GSH	Chow et al. 1975
rat HLA-Greenacres male	1.5-12.5 ppm x 1-5 h	↑neutrophil:lymphocyte ratio	Bobb and Fairchild 1967
rat C.R. Fischer male	1-2 ppm x 2 or 7 d	no blood chemistry changes	Cavender et al. 1977
guinea pig C.R. Hartley male	1-2 ppm x 2 or 7 d	no blood chemistry changes	Cavender et al. 1977
squirrel monkey	0.75 ppm x 4 h/d x 4 d	lung: ↑lipid oxidation ↓tocopherol ↑G6PD, GRase, LDH no effect MDH, SOD RBC's: ↑RBC fragility (H_2O_2) ↓GSH, (AChE) no effect LDH, G6PD (paired analyses)	Clark et al. 1978
rabbit	0.2 ppm x 4 h	↑osmotic fragility spherocytosis	Brinkman et al. 1964
rabbit New Zealand White male and female ~1 kg	0.4 ppm, 1 ppm 6 h/d x 5 d/wk x 10 mo	after ~105 d: ↓serum albumin ↑α- & γ-globulin no change total protein ↓appetite last 3 mo (effects greater in males)	P'an and Jegier 1976
rabbit	10 ppm x 1 h/wk x 6 wk	production of serum Ab's that reacted with ozonized egg albumin but not native ovalbumin	Scheel et al. 1959

(continued)

TABLE 11-1 (continued)

Species	Exposure Conditions	Observations[a]	Reference
Homo sapiens male	0.15 ppm x 1 h ± exercise 0.30 ppm x 1 h ± exercise	no effect NPSH, G6PD 6PGD, GRase, Hb	DeLucia and Adams 1977
Homo sapiens	0.2 ppm x 30-60 min	spherocytosis	Brinkman et al. 1964
Homo sapiens male	0.5 ppm x 165 min	↑osmotic fragility ↓AChE, GSH, G6PD ↑serum TBA, tocopherol, LDH	Buckley et al. 1975
Homo sapiens male (20) female (2) asthmatic	0.25 ppm x 2 h ± exercise	↑H_2O_2 fragility ↓GSH, AChE ↑G6PD, LDH no effect GRase, GPx, 23-DPG, Hct	Linn et al. 1978

[a] Definitions of abbreviations: Ab's, antibodies; AChE, acetylcholinesterase; 2,3-DPG, 2,3-diphosphoglycerate; GPx, glutathione peroxidase; GRase, glutathione reductase; GSH, reduced glutathione; G6PD, glucose-6-phosphate dehydrogenase; Hb, hemoglobin; Hct, hematocrit; LDH, lactate dehydrogenase; MDH, malate dehydrogenase; MetHb, methemoglobin; NPSH, nonprotein sulfhydryl; PK, pyruvate kinase; RBC's, red blood cells; 6PGD, 6-phosphoglycerate dehydrogenase; SOD, superoxide dismutase; TBA, thiobarbituric acid reactive products.

when, in primate studies, paired comparisons (each animal or human subject serves as its own control) were used in analyzing the data. These important points will be discussed later.

Our involvement in this kind of research began with the publication of research (Zelac et al. 1971) showing strikingly high levels of chromosome aberrations in circulating lymphocytes of Chinese hamsters exposed to approximately industrial tolerance levels of O_3. These findings raised questions regarding the carcinogenic potential of O_3 as well as the mechanisms that could be operative in affecting lymphocyte chromatin. Within the Medical Department at Brookhaven, C.J. Shellabarger's program offers the Sprague-Dawley rat mammary tumor model (Huggins et al. 1959) calibrated for tumor production by various radiations and chemical carcinogens (Shellabarger 1971, 1972; Shellabarger and Soo 1973; Shellabarger and Straub 1972). Hence, this model seemed suitable for a direct test of possible extrapulmonary carcinogenicity from O_3. Furthermore, mammary tissue seemed an appropriate "target tissue" considering our expectations that oxidizing lipids or their products might play a major role in amplifying any systemic cytotoxicity (Goldstein et al. 1969; Roehm et al. 1971). One such oxidation product, malonaldehyde, shows a weak mutagenic potential in the Ames screening test (Mukai and Goldstein 1976). In conjunction with the tumor studies, then, we began to look for biochemical markers indicating either (1) passage of oxidative products through the lung barrier or (2) other systemic changes attributable to O_3 (Borg and Shellabarger 1978).

INITIAL STUDIES

Using an O_3 exposure system constructed from a modified controlled atmosphere chamber, we first focused on finding evidence of lipid oxidation and directly related oxidative damage in red blood cells and mammary tissue: destruction of red cell membrane integrity, appearance of thiobarbituric acid reactive products (TBA) or fluorescence characteristic of iminopropene compounds, and changes in thin layer chromatography (TLC) patterns of extracted lipids. Subsequently, analyses for reduced glutathione (GSH), enzymes, and hemolysis were added to the list of parameters monitored. We found great variability in the resistance of individual rats and different batches of rats. ("Resistance" is here defined as the absence of observable changes in red cells; all exposed rats showed pulmonary lesions typical of O_3 toxicology.) Changes in the various parameters following acute exposures (e.g.: 3-8 ppm, 2-6 h, 1-3 d) were seldom observed in old rats (~>80 d), whereas rats exposed at ~40 d of age for only 1 d often showed dramatic responses in iminopropene fluorescence (Figures 11-1 through 11-3), oxidation products apparent on thin layer chromatographs (Figure 11-4), and depression of acetylcholinesterase activity. When such effects were manifested in red cells, they were short-lived (no more than 2 d), thereafter declining to approximately control level, then gradually increasing again when exposures were extended.

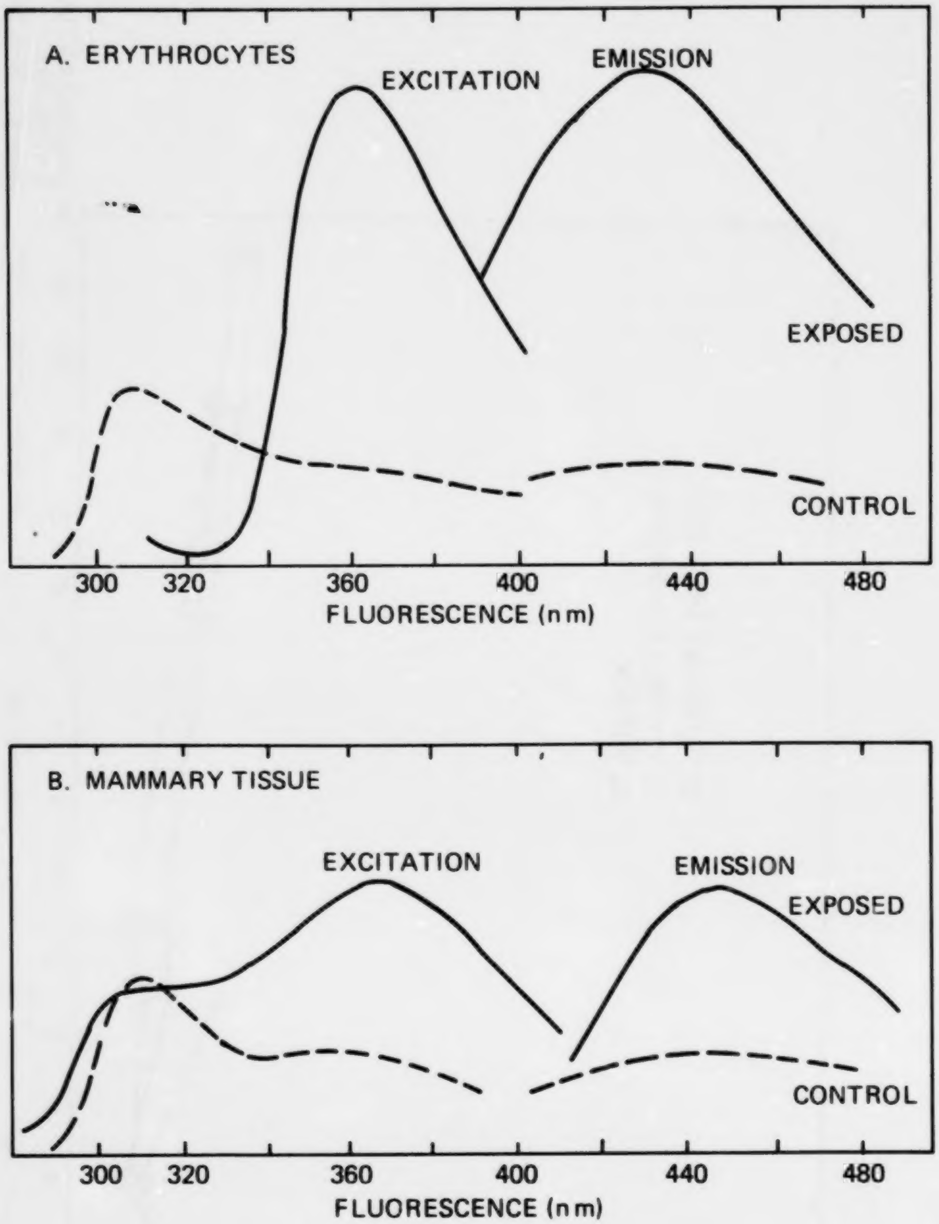

Figure 11-1. Typical iminopropene fluorescence patterns of erythrocyte (A) and mammary tissue (B) lipids from female Sprague-Dawley rats exposed to 2 ppm O_3 for 5 h.

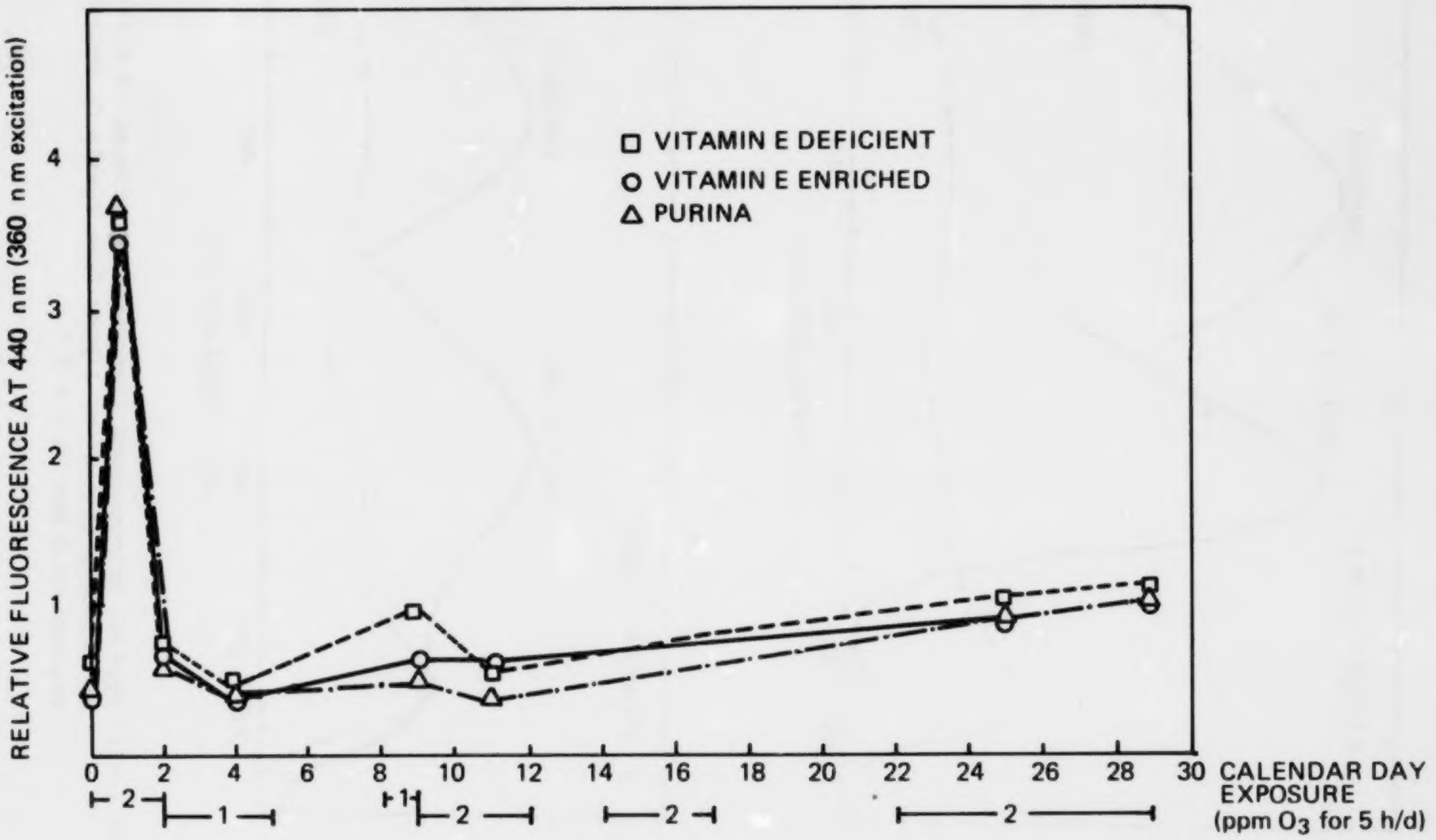

Figure 11-2. Iminopropene fluorescence in erythrocyte lipids from female Sprague-Dawley rats exposed chronically to O_3.

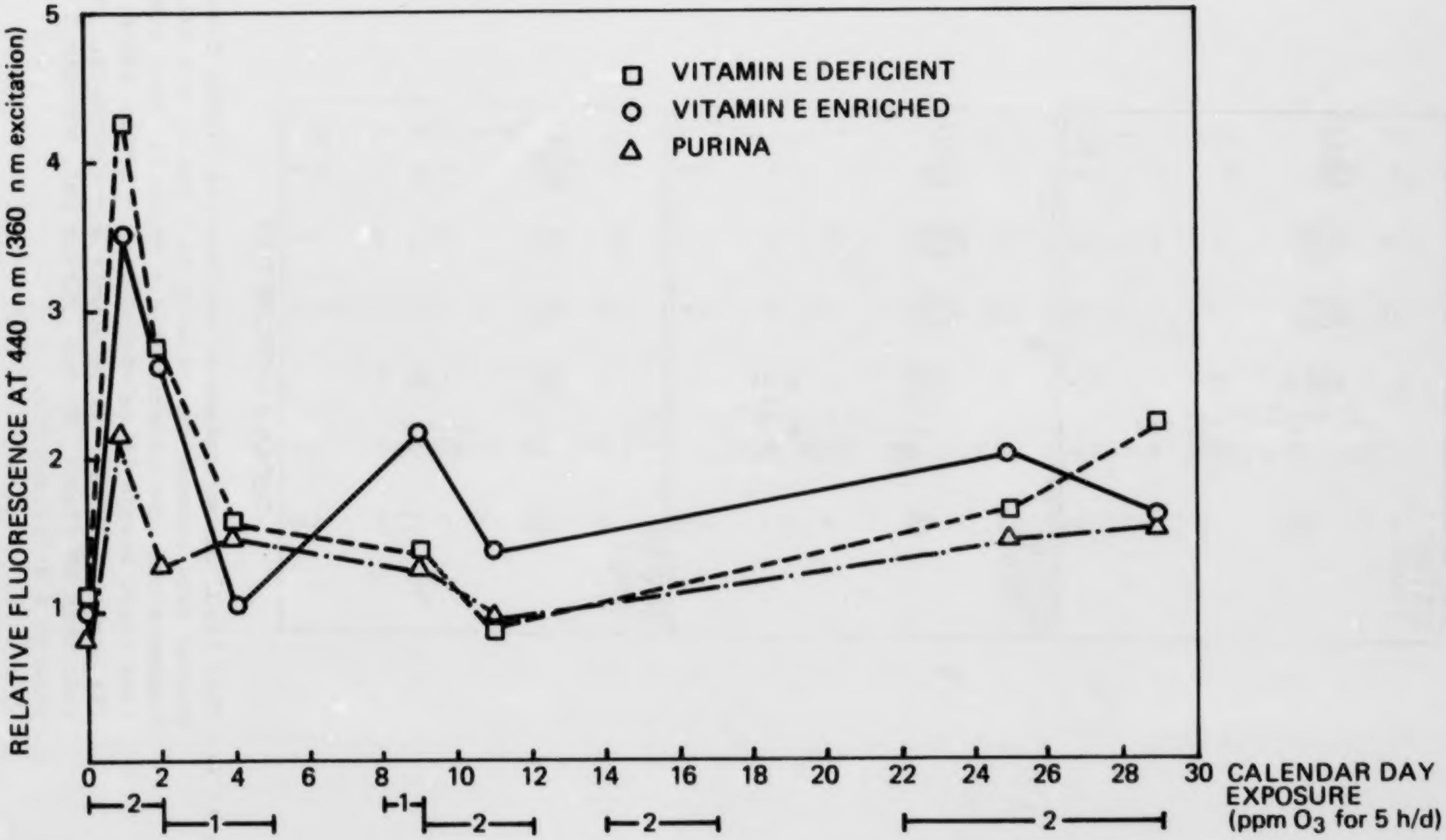

Figure 11-3. Iminopropene fluorescence in mammary tissue lipids from female Sprague-Dawley rats exposed chronically to O_3.

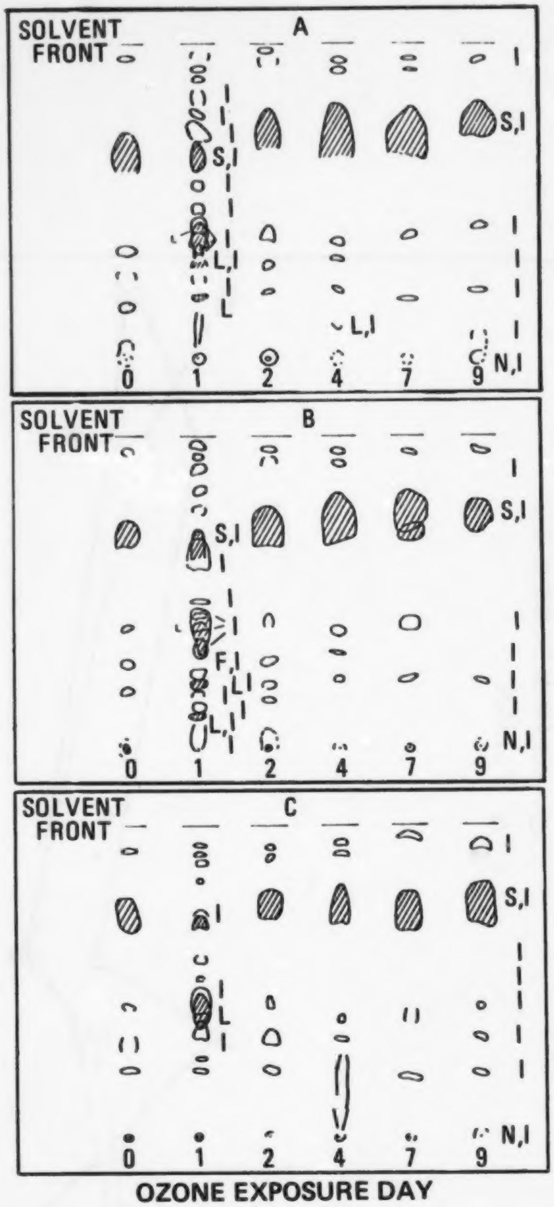
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Figure 11-4. Thin layer chromatograms of erythrocyte lipids from Sprague-Dawley rats exposed chronically to 2 ppm O_3 x 5 h/d. Solvent is hexane:ethyl ether:acetic acid = 80:20:1. Spot visualization (see right margin if unlabeled): I - iodine vapor; S - short UV; L - long UV; F - fluorescent; N - ninhydrin. Cross-hatching indicates UV light. Diets: Purina (A), vitamin E enriched (B), vitamin E deficient (C).

EFFECT OF DIETARY TOCOPHEROL

The difficulties we encountered in inducing measurable oxidative effects in red cells of rats fed the standard Purina Laboratory chow led us to consider dietary and antioxidant factors. Lab chow contains a relatively high level of tocopherol (for females more than twice and for males ~8 times the level necessary for maintaining reproductive capacity), so rats subsisting on it should be well protected against oxidants. Therefore, to find a more sensitive population in which potential systemic effects of O_3 would be more readily observed and defined, we used a synthetic "Draper" diet (Draper et al. 1964) to induce tocopherol deficiency in some rats before exposure. Other rats were maintained on the Draper diet supplemented with α-tocopherol (75 mg/kg chow, ~10 times the tocopherol level of standard chow).

Although the Draper diet is the classic diet most commonly employed in tocopherol deficiency studies, we found it to be less than satisfactory as a supporting diet. Females transferred to the diet (with or without supplementary tocopherol) immediately after weaning never grew properly or matured sexually, and hence could not be used for tumor studies (mammary tumor production in our model is hormone-dependent), and relatively mild acute exposures to O_3 (e.g., <2 ppm for 2 h) were occasionally lethal. As a compromise protocol, weanling rats were fed for 3 weeks on standard laboratory chow (by which time most had reached estrus) before transfer to one of the synthetic diets for 7 to 10 d (7 d was sufficient to attain a deficiency state, as measured by hemolytic susceptibility) and subsequent exposure to O_3.

Even so, deficiencies (or toxicants) other than tocopherol existed in the synthetic diets, and led to some anomalous response behavior, especially in the tocopherol-supplemented rats. A comparison of diet effects alone on control values shows some biochemical parameters (e.g., GSH, fragility, methemoglobin) to be consistent with antioxidant protection of extra tocopherol, whereas patterns of enzyme activities cannot be attributed entirely to tocopherol deficiency or supplementation (Table 11-2).

In extending our studies from acute to short-term chronic exposures, we included analyses of some red cell metabolic enzymes. Dietary factors represented a second set of variables. Our goal was to gain a clearer understanding of sequences of O_3 effects (if any) and to determine whether the apparent absence of lipid oxidation and glutathione destruction were real as opposed to reflecting enhanced intracellular decomposition and regeneration, respectively. We exposed rats on each of the three diets (Purina, Draper ± tocopherol) to 2.0 ppm O_3 for 5 h/d for 1 and 10 d. The animals were 50 d of age at first exposure.

As shown in Table 11-3, acute exposures under these conditions induced no changes in the red cell parameters measured, nor were effects much more marked after 10 d of exposure. What is interesting about the pattern of effects in the chronic exposures is that all statistically significant changes occurred in rats on the Draper synthetic diet--both with and without tocopherol (Table 11-4): decreased GSH (± tocopherol), increased TBA values and decreased osmotic fragility (+ tocopherol), decreased glucose-6-phosphate dehydrogenase

TABLE 11-2. DIET EFFECTS ON BIOCHEMICAL PARAMETERS IN RED BLOOD CELLS OF UNEXPOSED SPRAGUE-DAWLEY RATS

Test Parameter[a]	Diet			Probability (1-p) That Difference Between Diet Groups Is Not Due to Chance		
	Purina (P)	Vitamin E Enriched (E)	Vitamin E Deficient (D)	P-E	P-D	E-D
GSH (mg %)	75.16 ± 0.86	88.13 ± 3.24	68.47 ± 2.32	1.00	1.00	1.00
TBA[b]	6.71 ± 0.76	8.27 ± 0.98	8.97 ± 1.46	0.99	0.99	0.82
osmotic fragility[c]	0.476 ± 0.003	0.461 ± 0.006	0.470 ± 0.007	0.96	0.81	0.85
G6PD (units)	13.27 ± 0.04	12.42 ± 0.62	10.09 ± 0.44	0.98	1.00	1.00
AChE (units)	11.86 ± 0.48	11.56 ± 0.21	11.35 ± 0.77	0.88	0.90	0.72
MetHb (%)	4.81 ± 0.34	2.72 ± 0.35	3.60 ± 0.62	1.00	~1.00	0.99

[a] Definitions of abbreviations: TBA, thiobarbituric acid reactive products; MetHb, methemoglobin; GSH, glutathione; AChE, acetylcholinesterase; G6PD, glucose-6-phosphate dehydrogenase. Values given are mean ± standard deviation.

[b] Moles x 10^{-6} thiobarbituric acid products/ml packed cells.

[c] % NaCl solution giving 50% hemolysis.

TABLE 11-3. BIOCHEMICAL CHANGES IN RAT RED BLOOD CELLS FOLLOWING ACUTE EXPOSURE TO OZONE[a]

Test Parameter[b]	Diet								
	Purina			Vitamin E Enriched			Vitamin E Deficient		
	Control	Exposed	p[c]	Control	Exposed	p[c]	Control	Exposed	p[c]
TBA[d]	6.64 ± 0.53	7.14 ± 0.91	NS	6.23 ± 1.16	5.73 ± 0.82	NS	5.77 ± 0.48	5.85 ± 0.69	NS
MetHb (%)	4.35 ± 0.60	4.49 ± 0.30	NS	3.88 ± 1.16	3.53 ± 0.53	NS	4.27 ± 0.67	4.57 ± 0.60	NS
osmotic fragility[e]	--	--		0.526 ± 0.020	0.503 ± 0.013	NS	0.533 ± 0.05	0.527 ± 0.03	NS
GSH (mg %)	79.3 ± 14.8	82.8 ± 13.0	NS	89.77 ± 6.22	85.86 ± 3.44	0.06	115.3 ± 2.8	116.4 ± 7.6	NS
AChE (units)	13.6 ± 1.5	14.2 ± 2.3	NS	14.1 ± 3.7	13.2 ± 2.5	NS	12.4 ± 1.6	12.5 ± 2.0	NS
G6PD (units)	8.2 ± 0.9	8.2 ± 0.9	NS	8.6 ± 1.5	9.0 ± 1.2	NS	11.5 ± 0.6	11.7 ± 1.1	NS

[a] Purina diet: 3 ppm O_3 x 4 h. Vitamin E Enriched and Deficient diets: 2 ppm O_3 x 4 h.

[b] Definitions of abbreviations: TBA, thiobarbituric acid reactive products; MetHb, methemoglobin; GSH, reduced glutathione; AChe, acetylcholinesterase; G6PD, glucose-6-phosphate dehydrogenase. Values given are mean ± standard deviation.

[c] NS = not significant ($p > 0.10$ in one-tailed t-test).

[d] Moles x 10^{-6} thiobarbituric acid reactive products/ml packed cells.

[e] % NaCl solution giving 50% hemolysis.

TABLE 11-4. BIOCHEMICAL CHANGES IN RAT RED BLOOD CELLS FOLLOWING CHRONIC EXPOSURE TO OZONE[a]

Test Parameter[b]	Diet								
	Purina			Vitamin E Enriched			Vitamin E Deficient		
	Control	Exposed	p[c]	Control	Exposed	p[c]	Control	Exposed	p[c]
TBA[d]	6.71 ± 0.76	7.43 ± 1.21	NS	8.27 ± 0.98	9.39 ± 1.49	0.06	8.97 ± 1.46	9.48 ± 2.10	NS
MetHb (%)	4.81 ± 0.34	4.28 ± 0.76	0.10	2.72 ± 0.35	2.77 ± 0.30	NS	3.60 ± 0.62	3.81 ± 0.47	NS
osmotic fragility[e]	0.476 ± 0.03	0.474 ± 0.002	NS	0.461 ± 0.006	0.449 ± 0.006	-(0.06)	0.470 ± 0.008	0.471 ± 0.000	NS
GSH (mg %)	75.16 ± 0.86	75.35 ± 3.31	NS	88.13 ± 3.24	69.63 ± 5.27	0.005	68.47 ± 2.32	62.21 ± 4.13	0.005
AChE (units)	11.86 ± 0.48	12.10 ± 0.35	NS	11.56 ± 0.20	11.77 ± 0.99	NS	11.35 ± 0.77	11.11 ± 1.15	NS
G6PD (units)	13.27 ± 0.04	14.49 ± 0.47	NS	12.42 ± 0.62	12.24 ± 1.08	NS	10.09 ± 0.44	9.21 ± 0.63	0.01
Heinz bodies (%)	0.10 ± 0.00	0.10 ± 0.00	NS	0.50 ± 0.28	0.60 ± 0.36	NS	1.40 ± 0.28	2.20 ± 0.00	0.03

[a] 2 ppm O_3 x 4 h/d x 10 d.

[b] Definitions of abbreviations: TBA, thiobarbituric acid reactive products; MetHb, methemoglobin; GSH, reduced glutathione; AChE, acetylcholinesterase; G6PD, glucose-6-phosphate dehydrogenase. Values given are mean ± standard deviation.

[c] NS = not significant (p > 0.10).

[d] Moles x 10^{-6} thiobarbituric acid reactive products/ml packed cells.

[e] %NaCl solution giving 50% hemolysis.

(G6PD) and increased Heinz bodies (- tocopherol). The seemingly anomalous decrease in fragility after O_3 exposure in the supplemented rats may be explained by observations of substantial hemolysis in blood drawn from vitamin E supplemented rats; washed cells used for this test could be a residual, more resistant population.

DISCUSSION

From this inconsistent pattern of changes alone (not to mention much of our early experience with acute and other short-term chronic exposures) it is tempting to conclude that cytotoxic or oxidative potential from O_3 is not transferred from lung tissue to circulating red cells. However, we are not ready to dismiss the issue of systemic effects for three primary reasons:

1. TLC patterns of lipids extracted from red cells and mammary tissue indicate oxidative degradation of lipids;
2. Unpublished data from our lab and Dr. Drew's bring into question the utility of red cell studies in screening for oxidant systemic effects;
3. Rats, and perhaps all rodents, are unsuitable as models for human response to atmospheric oxidants.

The following paragraphs consider each of these points in more detail.

Evidence for Oxidative Degradation of Lipids

After nearly every short-term chronic exposure, some degree of oxidative response was apparent, but the pattern of response varied between batches of animals and with age, exposure level, etc. The only effect that occurred repeatedly and consistently with all diet groups, batches, and exposures (including high-level acute exposures) was changes in TLC patterns of extracted lipids.

New TLC spots typical of those expected from a variety of oxidation products appeared in the nonpolar fractions of lipids extracted from red cells (Figure 11-5). The classes of lipids that seem to have been involved are cholesteryl esters, diglycerides and triglycerides, and free fatty acids (and perhaps their esters), indicating glyceride hydrolysis and fatty acid oxidation. Tocopherol showed little effect.

In contrast, in mammary tissue the phospholipid and polar lipid fractions were most affected (Figure 11-6), and the TLC changes were cumulative with exposure time (Figure 11-7). From comparisons with pure standards, it seems that, in mammary tissue, the phospholipids phosphatidylethanolamine, phosphatidylcholine (lecithin), phosphatidylserine, and phosphatidylinositol are the most likely targets. The changes are consistent with fatty acid oxidation, hydrolysis to lysolecithins and phosphatic acids, and formation of iminopropene fluorescent products from reaction of fatty acid carbonyls with amino groups.

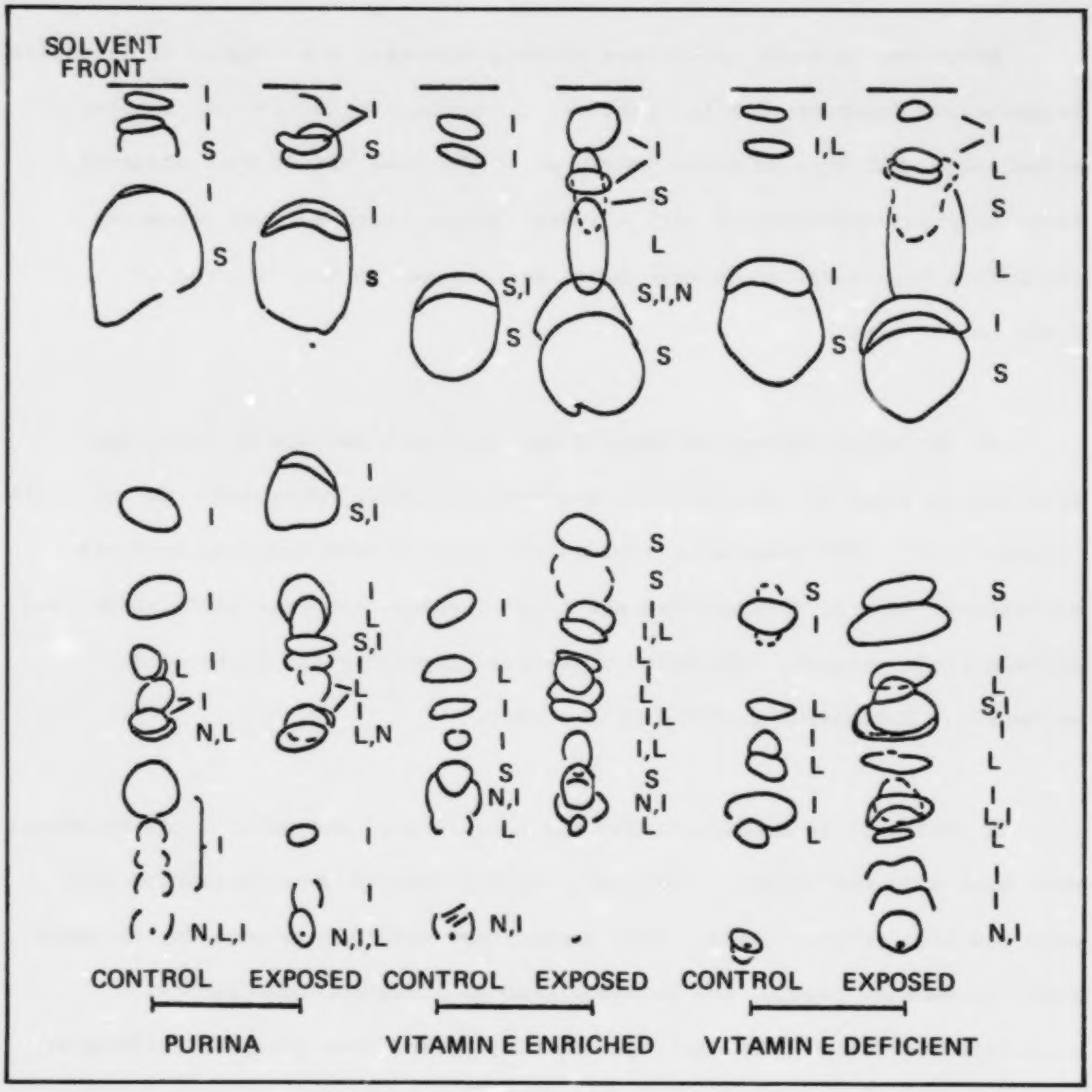
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Figure 11-5. Thin layer chromatograms of erythrocyte lipids from female Sprague-Dawley rats exposed to O_3 (2 ppm x 4 h x 10 d). Solvent is hexane:ethyl ether:acetic acid = 80:20:1. Spot visualization: I - iodine vapor; S - short UV; L - long UV; N - ninhydrin.

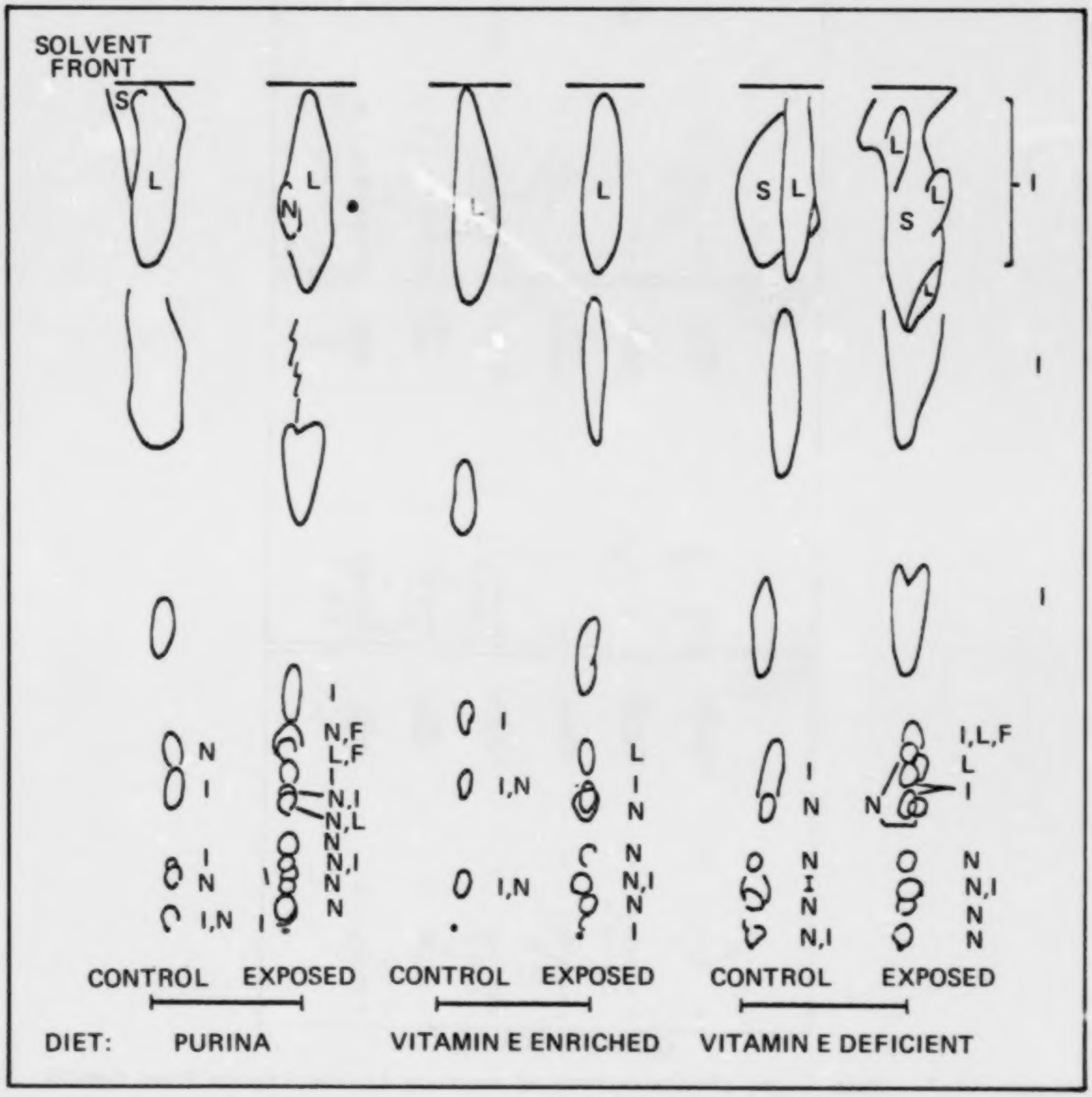
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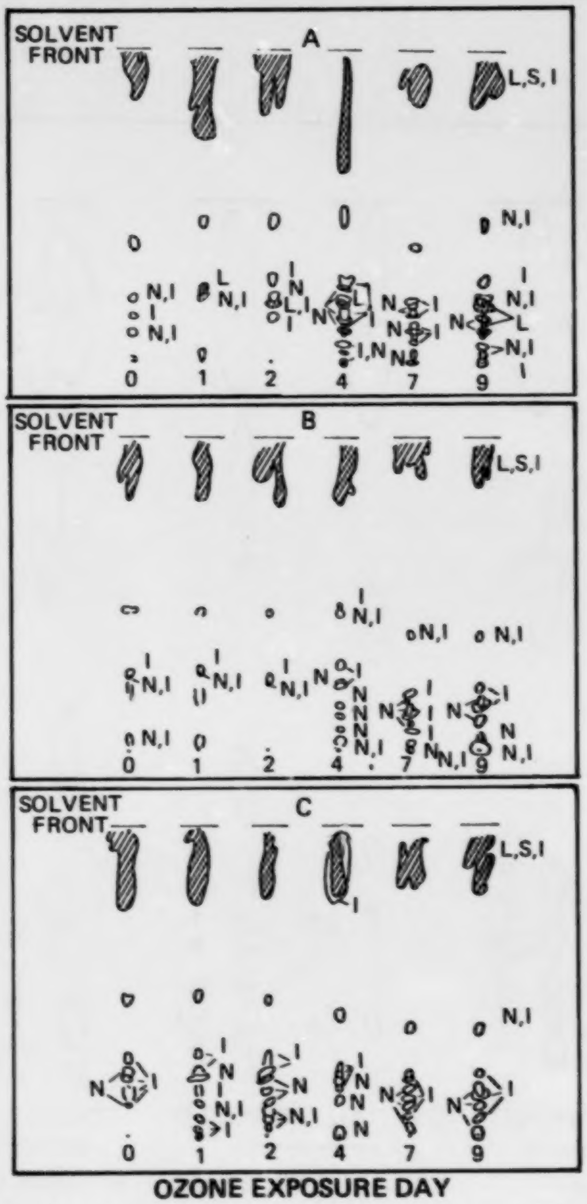
Figure 11-6. Thin layer chromatograms of mammary tissue lipids from female Sprague-Dawley rats exposed to O_3 (2 ppm x 4 h x 10 d). Solvent is chloroform:methanol:acetone:acetic acid:water = 65:10:20:10:3. Spot visualization: I - iodine vapor; S - short UV; L - long UV; F - fluorescent; N - ninhydrin.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OZONE EXPOSURE DAY

Figure 11-7. Thin layer chromatograms of mammary tissue lipids from female Sprague-Dawley rats exposed chronically to 2 ppm O_3 x 5 h/d. Solvent is chloroform:methanol:acetone:acetic acid:water = 65:10:20:10:3. Spot visualization (see right margin if unlabeled): I - iodine vapor; S - short UV; L - long UV; F - fluorescent; N - ninhydrin. Cross-hatching indicates UV light. Diets: Purina (A), vitamin E enriched (B), vitamin E deficient (C).

These changes show clearly that oxidative potential initiated in the lung can be carried to distant sites. Whether these specific changes are functionally meaningful is another matter, since they cannot be correlated with any of the other parameters measured. Nevertheless, they do indicate latent damage which, under our exposure conditions, is either (1) not sufficiently extensive to affect cell functioning, or (2) rapidly repaired (e.g., by the spleen's clearing of damaged red cells).

The Dynamic Nature of the Systemic Response to Oxidants

The concept of latent damage brings us to some unreported observations in our laboratory suggesting that systemic responses to oxidants and related secondary stress are dynamic. From these observations we can infer that "spot-checking" a few parameters for evidence of deleterious effects might not yield an accurate picture of what is happening *in vivo*.

A few reports in the literature have considered red cell morphology to be affected by O_3, noting spherocytosis and crenation of red cells (Larkin et al. 1978) as well as reticulocytosis and changes in the proportional distributions of blood cell types (Bobb and Fairchild 1967). We have noted but not yet quantified similar changes. In our studies, spherocytosis seemed most common for low-level exposures whereas formation of echinocytes (crenated or spiny cells) was quite marked at high-level acute exposures or after chronic exposures. Each of these types of cells denotes latent damage that alters cell response to the osmotic environment without, for the most part, affecting cell function. We have also seen a variable reticulocytotic response. When reticulocytosis seemed most marked (with attendant increases in hematocrit), biochemical measures showed the greatest change and the animals themselves showed the greatest respiratory distress. Thus, we are left with several questions: Is the reticulocytosis a direct response to red cell damage *in vivo*, or is it a secondary reaction to pulmonary pathology? Since enzyme activities in young erythrocytes are generally higher than in mature cells, is it possible that the apparent lack of observable changes results from larger numbers of reticulocytes counterbalancing decreased activities of defective cells? To what extent do these types of latent damage indicate the presence of a rat response threshold below which "systemic" effects of O_3 are detectable only in hypersensitive populations, such as in tocopherol or other nutrient deficiencies? We are currently looking for answers to these questions.

Consider, also, that few studies have focused on tissues other than blood, presumably on the assumption that any extrapulmonary effects will be first noticeable in the bloodstream. In some preliminary studies by Dr. Drew's group (Costa and Drew 1980), rats exposed to sulfur dioxide for only a few hours showed a reproducible destruction of sulfhydryl groups in the lung and liver but not in blood. While extrapolation would be premature, these findings certainly raise questions about the utility of red cell studies in screening for systemic effects of oxidants.

Nonsuitability of Rats as Models for Human Response to Oxidants

This brings us to the last point: the nonsuitability of rats (and perhaps all rodents) as models for human response, either pulmonary or systemic, to O_3 and oxidant gases. The difficulties we have encountered in producing in rats a consistent pattern of measurable systemic damage from O_3 similar to that observed in other species, coupled with known physical and biochemical/metabolic characteristics of the rat, lead us to contend that the rat is in fact a poor surrogate for humans. Three lines of reasoning support this contention.

First, there are significant differences in respiratory structure between rodents and primates. Rats are obligate nasal breathers; thus O_3 uptake in the upper airway is greater than if breathing were by mouth (as in, for example, humans who are exercising or who have nasal obstruction). Unlike primates, rats do not have well developed respiratory bronchioles (the transition phase between the terminal bronchiole and alveolar ducts) (Mellick et al. 1977; Castleman et al. 1977). Both of these factors contribute to the decreased distribution of oxidant gases to respiratory exchange tissues in the rodent. Miller et al. (1978) developed mathematical models of transport and removal of oxidant gases (O_3 and nitrogen dioxide) in lungs of different species. These authors predicted the following relative respiratory bronchiolar doses (assuming tracheal doses of >0.05 ppm): man, 100; rabbit, 80; guinea pig, 40. Relative comparisons of lung morphology and structure would place rats on the lower end of this scale. The net implication is that, for identical doses, much less O_3 reaches peripheral lung cells and the gas/blood barrier in rats than in humans. In addition, the GSH peroxidase system for detoxifying and preventing accumulation of oxidative products in lung tissue normally operates at a much higher level in rats than in primates--e.g., four times that in monkeys (Chow et al. 1975)--and is also more readily stimulated by oxidant challenge. Thus, neither primary oxidant (O_3) nor secondary toxic products such as peroxides would be expected to pass into blood at levels comparable to those in humans and other primates.

"Antioxidative" defense mechanisms in red cells are also much more active in rats than in man. Kurian and Iyer (1977), for example, have shown that rats and other rodents are much less susceptible than man and other primates to stress with the oxidant acetylphenyl hydrazine (APH) (Figure 11-8). Heinz body production is also correspondingly decreased. Higher levels of GSH, GSH peroxidase, and G6PD protect against even intense oxidative stress while efficiently regenerating active levels of intracellular reducing agents (Table 11-5). Thus, in comparison to human red cells, rat red cells should be better able to cope with any O_3 stress.

Finally, there are problems with statistical treatment of data necessary to show "significant" differences between exposed and control values. In studies citing significant O_3 effects on red cell enzyme levels in monkeys (Clark et al. 1978) and humans (Buckley et al. 1975; Linn et al. 1978), the actual differences in activities were small. (For example, Table 11-6 presents some results of the Linn et al. study.) However, the data were

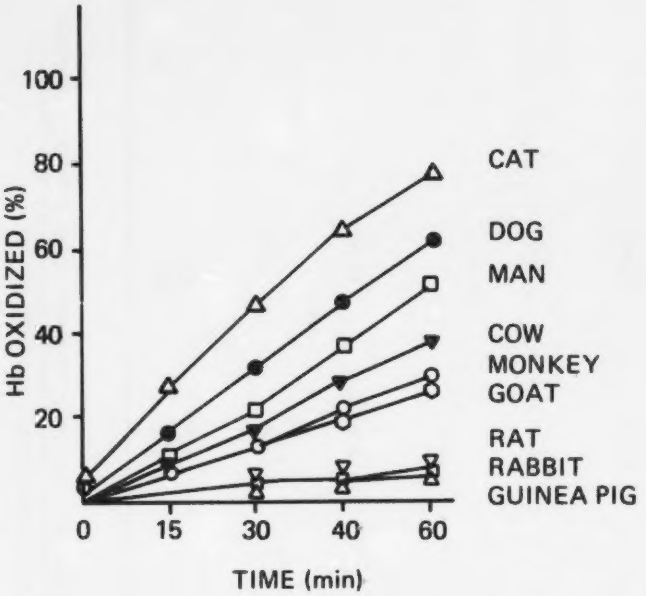

Figure 11-8. Species differences in sensitivity to oxidative (APH) stress. A 10% suspension of washed erythrocytes in Ringer phosphate was treated with APH (2 mg/ml) at 38°C; at 15-min intervals 0.5-ml aliquots were withdrawn, diluted with 5 ml ice-cold water, and MetHb estimated. Data from: Kurian and Iyer (1977).

TABLE 11-5. SPECIES DIFFERENCES IN LEVELS OF SOME PROTECTIVE ENZYMES FOLLOWING STRESS WITH ACETYL PHENYLHYDRAZINE[a]

Parameter[b]	Rat	Man	Monkey
SOD (units)	52	30	28
GPx (μM GSH/g Hb/min)	122	12	5
Catalase (K x 10^3)	8.6	36.5	38.7

[a] Data from: Kurian and Iyer (1977).

[b] Definitions of abbreviations: GPx, glutathione peroxidase; GSH, reduced glutathione; Hb, hemoglobin; SOD, superoxide dismutase.

TABLE 11-6. EFFECTS OF OZONE IN ASTHMATICS[a]

Variable[b]	Sham[c]	Odor-Sham[c]	O_3 Exposure[c]	F(2,42)[d]	p[d]	Change (%)
Hb (g/100 ml)	14.99 ± 0.96	14.97 ± 0.94	14.88 ± 0.99	5.2[illegible]	0.009	0.7
Hct (%)	44.4 ± 2.9	44.6 ± 2.7	44.2 ± 2.7	1.83	NS	1
H_2O_2 fragility	24.1 ± 7.0	25.0 ± 6.9	26.8 ± 7.2	7.46	0.002	11
GSH	30.8 ± 5.4	28.7 ± 5.4	29.3 ± 5.0	5.53	0.007	5
AChE	22.2 ± 2.5	21.5 ± 2.4	20.9 ± 2.5	24.94	<0.001	6
G6PD	5.12 ± 1.06	5.45 ± 1.16	5.67 ± 1.20	9.98	<0.001	11
LDH	104.0 ± 12.8	108.4 ± 16.1	111.4 ± 17.0	4.82	0.013	7
GRase	2.64 ± 0.79	2.66 ± 0.78	2.68 ± 0.74	0.89	NS	-
GPx	10.87 ± 3.05	10.67 ± 2.73	10.99 ± 2.54	0.57	NS	3
2,3-DPG	14.30 ± 1.67	14.52 ± 1.78	14.58 ± 2.53	0.50	NS	2

[a] Adapted from: Linn et al. (1978) (Table 6). Exposure = 0.25 ppm O_3 x 2 h. N = 22.

[b] Definitions of abbreviations: AChE, acetylcholinesterase; 2,3-DPG, 2,3-diphosphoglycerate; GPx, glutathione peroxidase; GRase, glutathione reductase; GSH, reduced glutathione (red blood cell); G6PD, glucose-6-phosphate dehydrogenase; Hb, hemoglobin concentration; Hct, hematocrit; H_2O_2 fragility, red blood cell fragility expressed as % hemolysis when incubated with 2% hydrogen peroxide solution; LDH, lactate dehydrogenase.

[c] Values shown are mean ± S.D. Pairwise significant differences are indicated by solid arrows for $p < 0.05$; by dashed arrows for $p < 0.01$.

[d] Overall variation is indicated by the F statistic (degrees of freedom in parentheses) and the p value. NS = not significant.

obtained as paired comparisons (comparisons of measures on the same subject before and after exposure) rather than as comparisons between exposed and nonexposed populations. We took the post-exposure mean and standard deviation data from some paired-comparison studies and applied several other standard statistical tests of group differences. As we had suspected, under these conditions--conditions commonly used in small animal studies--we found no statistical differences, thus illustrating the power of paired comparisons as a statistical tool.

In small animals, the problems are twofold: (1) paired comparisons are not physically possible; (2) differences in individual animal responses give group variances that are too large to allow statistical detection of small but probably real group differences. If, then, the issue of O_3 effects in red cells is largely a "numbers game," the rules are predesigned for "no effects" in any small animal study that doesn't involve very large numbers of animals or show large differences in group mean values. With respect to the latter, comparisons of exposed and control lung tissue (the first site of interaction with oxidant gases) in rats and other species have shown 25% or greater differences in, for example, enzyme or GSH levels. But comparable effects in red blood cells--especially the red blood cells of rodents--would not be expected and in fact have not been seen, even in the presence of superimposed stress such as tocopherol deficiency.

This analysis does not imply that the use of rats and other rodents in oxidants research should be discontinued. However, the considerations outlined here urge strong caution in the use of rats as surrogates for human beings. Especially in the case of extrapulmonary effects, it is extremely doubtful whether "no effect" observations in rats can be credibly extrapolated to predict "no" human responses.

SUMMARY AND OVERVIEW OF CURRENT RESEARCH

In summary, acute and chronic exposures of rats to relatively high levels of O_3 have failed to provide evidence for statistically significant extrapulmonary effects in red cells except for oxidation in component lipids. Hematological observations and trends in GSH and enzyme changes, however, suggest the possibility of a response threshold not reached in animals exposed under our experimental conditions. Observations of damage to lipid and GSH in mammary and liver tissues, respectively, indicate the potential even in rats for systemic effects at sites distant from the lung--effects that have been largely unexplored.

Finally, although laboratory rats are quite useful for research purposes, they may not be appropriate or adequate models for directly predicting human response to gaseous oxidative pollutants. We must identify an animal having a respiratory structure comparable to that of the human but which is less expensive than primates and large enough to allow sequential tissue sampling and paired before-and-after comparisons.

Efforts to respond to these research needs are currently underway within the Medical Department at Brookhaven, where we are (1) exposing rats to O_3 for longer periods (up to 67 d) and correlating measures of lung function, biochemistry, and pathology with red cell biochemistry and metabolism, hematology, serum measures of hepatic function, and cytogenetic end points; and (2) exposing sheep to oxidant gases and investigating mechanisms of a wide variety of effects. In the latter system, paired before-and-after comparisons as well as selective exposure of single lobes (using another nonexposed lobe in the same animal as the control) are possible. Also, the species is sufficiently large to allow simultaneous studies of systemic functional or biochemical, immunological, and pulmonary function and biochemistry studies in a single animal. Such coordinated studies should provide considerable insight into the total and overall effects of oxidative pollutants, the mechanisms responsible, and the relationship between effects on different body systems.

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WORKSHOP COMMENTARY

Question: Did you test for hemolysis in these studies?

K. M. Schaich: Yes.

Question: And you did not find hemolysis?

K. M. Schaich: We tried a number of hemolysis stress tests and finally used osmotic hemolysis--differential response to salt--because it gave the most consistent results. However, this is perhaps not the right measure for hemolysis. Osmotic hemolysis reportedly is related to the sphericity of the cells (the amount that they are puffed up). It results from pressure from the inside. Thus, in the absence of overt damage to the cell membrane or gross alterations to intracellular chemistry, observations of osmotic stress effects are unlikely.

Also, the rat red cells seem to be much more resistent to osmotic stress than human erythrocytes.

Question: What is the possibility that you have a red cell subpopulation which is so sensitive that it breaks down, and is accordingly not tested?

K. M. Schaich: That is precisely why I mentioned our findings with tocopherol-supplemented rats. We have not yet been able to do the hematological studies in a controlled quantitative way at the same time as the biochemical studies. However, in the supplemented rats we could never draw blood without substantial hemolysis; thus, we suspect a supersensitive population of cells in those rats. The same kind of hemolysis did not occur in handling the other rats, but those animals' spleens may have cleared the damaged cells more efficiently and rapidly.

I can't give you a confirmed answer. I suspect that we may in fact be looking at a more resistant, residual population, but until we finish the current study in which we are monitoring hematological and biochemical changes, I cannot say anything more definite.

Question: In your interesting results on extrapulmonary effects, are you able to control for lung injury as a possible nonspecific kind of extrapulmonary effect?

K. M. Schaich: We cannot. That is one of the problems of this type of animal study. To my knowledge, in the literature there have been no reports directly comparing and correlating pulmonary and extrapulmonary effects of oxidant gases. Recently, there have been some studies which monitored what was happening morphologically in the lung with what was happening biochemically in the bloodstream, but I think the correlations were more limited than the implications of your question. We have always been concerned that the changes we observe in red cells may be secondary responses to infection rather than primary effects of O_3. It is precisely this consideration that led us, in our current studies, to use specific pathogen free animals (thereby eliminating endemic pulmonary infections) and to simultaneously monitor hematological changes (if any), red cell and serum biochemistry, and lung function and pathology. In this way we hope to be able to differentiate primary and secondary effects.

Question: What was the age of the rats when you started the studies?

K. M. Schaich: In the chronic studies, the rats were ~50 d of age when we started the exposures, and that bothers me. Age at initial exposure is one reason why we handled our deficient diets (the Draper diets) in a manner different from that usually reported in the literature. Normally, rats are maintained on lab chow for a month or two to allow them to grow and establish approximately adult weight before transferal to the deficient diets.

Considering our earlier results, however, we were afraid we would miss potential early effects if we followed such a procedure. We would have preferred to maintain rats on the Draper diets from the weanling age (19-21 d) so that they would be deficient by 40 to 42 d--the age at which we saw the early responses in rats on lab chow. Regardless of diet, animals did not show "first day" or acute effects if they were older than ~45-48 d.

There is a marked age dependence in the response of rats to various radiations and chemicals. This age dependence is at least partly related to hormone levels. Shellabarger showed in his mammary tumor model that there is a critical period between ~45 and ~60 d where hormonal changes proceed so rapidly that the overall response of the rats to systemic challenges is altered.

I cannot pinpoint how potential responses were affected by our regimen.

H. P. Witschi: Would you expand on your comment that rats are not appropriate experimental animals for pulmonary studies?

K. M. Schaich: I don't think rats are adequate models for human response, since blood studies in humans have consistently demonstrated some effects in red cells and red cell enzymes while rat studies have not. Compared to humans, I don't expect as much O_3 or oxidative potential to get through the lung to the blood because of the rat lung structure and because of the biochemical protective mechanisms which are much more active in rat lung.

H. P. Witschi: Oxygen readily diffuses through membranes. How do you know that O_3 isn't acting similarly, without reacting in the membrane?

K. M. Schaich: I don't think that O_3 itself gets into the blood; the more likely effective toxic agent(s) is some breakdown product of O_3 or some secondary oxidation product. There has been a long-standing dispute regarding the disposition of O_3 in the respiratory tree and what happens to O_3 when it traverses membranes (if it does). Ozone is so reactive that when it contacts anything (e.g., surfactant), it decomposes or interacts immediately with a sensitive group. Therefore, in the rat it is unlikely that O_3 itself, having to traverse the nasal passages as well as the upper respiratory tree, would ever get into distal portions of the lung. Oxidation products such as superoxide anion may get through to the air/blood interface, but that species is also very reactive. I think it is more likely that secondary oxidation products of lipids in the membranes--hydroperoxides or epoxides or product aldehydes--provide the mobile and more stable oxidative potential that may be picked up by the blood and carried on to initiate subsequent damage.

Comment: Ozone is more reactive than oxygen, and oxygen doesn't diffuse very well through the upper airway. That region, therefore, is not an important locus for gas exchange. As others have shown in terms of the depth to which one can see damage in the lung following cytotoxic O_3 exposures, damage hardly gets below the basal membrane, so in thick tissues not much gets through. Ozone and gas diffusion in the lungs is quite different than in cell suspensions *in vitro*.

J. A. Graham: Using a different systemic parameter and agents other than O_3, we've found that the female is much more sensitive than the male. Were you using males or females?

K. M. Schaich: We were using females in the beginning; we are using males now. We started with Sprague-Dawley rats and now we're using Fischer rats. For the first couple of years we used "scrap rats" from wherever we could get them.

When we did have male rats, our impressions were that they were less sensitive. But they were usually also older, so that confounds the issue.

Comment: Some of these parameters are reminiscent of aging red cells. Have you determined the age distributions of the red cells you sampled? Were they comparable?

K. M. Schaich: No, we had no hematological backup in the early studies. You can see, however, there is a long list of things that we're beginning to follow up now. In a study that is just beginning, we will look directly at a variety of hematological parameters in order to understand the red cell population being monitored. If we don't have the same red cell populations and type distributions in exposed and normal rats, then obviously we should see some differences in the enzyme responses. We are not presently equipped, however, to measure red cell kinetics and turnover, which would provide a more direct answer to your question.

E. Hu: Are you testing the effects of oxidants on the tumorous animal, too?

K. M. Schaich: Shellabarger did those studies and we worked in conjunction with him. Those studies were essentially negative. There were some borderline positive responses but nothing consistent.

Question: There are two forms of glutathione peroxidase. Which form were you talking about?

K. M. Schaich: I don't know that we differentiate; we use standard glutathione peroxidase assays for the red blood cells as outlined by E. Beutler [1975. Red Cell Metabolism: A Manual of Biochemical Methods. Grune and Stratton, New York].

Question: How do you measure? Do you use hydrogen peroxide?

K. M. Schaich: No, t-butyl hydroperoxide.

Question: Because there are different levels of the peroxidase and other materials that might decompose O_3 in the blood, you're assuming that it is in fact the O_3 that's causing the problem. But maybe the decomposition products are effecting the damage.

K. M. Schaich: I don't think the O_3 is causing damage directly in the red cells. As I said before, I believe it to be more likely that ozonides or other oxidation products carry the damage potential from the lungs to other tissues. That is why we initially looked only for oxidation effects, especially in lipids.

Question: Since peroxidase activities are higher in rats than in humans or in primates, is it possible that you saw even more effect, because breakdown products enter the bloodstream faster?

K. M. Schaich: No. Hydroperoxides are decomposed by peroxidases to products with lower oxidation potential and generally more hydrophobic character. These products, therefore, are less likely to be picked up by the bloodstream, and are less damaging if they are. More rapid peroxide decomposition, furthermore, means that lower concentrations of the toxic hydroperoxides will be available for transport by the bloodstream. Hydrogen peroxide and lipid hydroperoxides that do get into circulation provide quite active substrates for the iron in the blood, forming an in vivo Fenton-like system which produces, at a minimum, the more reactive and cytotoxic hydroxy and alkoxy radicals. Thus, in terms of potential damage it should be of advantage to keep hydroperoxide levels as low as possible.

Question: These agents occur in the blood, and your measurements were in the blood?

K. M. Schaich: Yes. Let me restate my reasons for contending that rats are poor models for providing data which may be directly extrapolated to man--at least in terms of pulmonary and systemic oxidant effects. First: Rat lung tissue has a substantially greater capacity than does the human lung for decomposing oxidation products formed during normal metabolism or after exposure to gaseous or other oxidants. Thus, lower concentrations of the most potently damaging products are available to be picked up and circulated by the blood. Consequently, initial challenge to circulating red blood cells is lower in rats than in man. Second: Levels of glutathione peroxidase in red cells are much higher in rats than in man. Since glutathione peroxidase is located at the membrane and acts on organic peroxides as well as hydrogen peroxide, effects in rat red cells will be counteracted at the membrane level--at the site of initial oxidant reaction. In contrast, in man higher levels of catalase alone are ineffective in counteracting sustained oxidant stress, especially at the membrane level. Damage to the membrane allows cytotoxic agents to be absorbed or otherwise taken up by the cell, thereby affecting metabolic machinery and other components inside the cell. Thus, the site of major reactions and red cell damage are quite different for rats as compared to man. As a consequence, patterns of response to oxidant challenge would not be expected to be comparable.

12. PATHOGENESIS OF CHRONIC LUNG DISEASE: THE ROLE OF TOXICOLOGICAL INTERACTION

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INTRODUCTION

It is known that simultaneous or successive exposure to two or possibly several toxic agents will sometimes elicit a biological response that is considerably more severe than the effects produced by one chemical alone (National Research Council 1980). To consider toxicological interactions should be an important element in attempts to estimate and evaluate the health effects of air pollution. These attempts require an understanding of the nature and mechanism of each interaction.

Our group recently developed an experimental model to help describe and analyze how an acute interaction between two toxic agents produces a chronic form of lung damage. The two compounds are the antioxidant butylated hydroxytoluene (BHT) and oxygen (O_2). We hope that it will be possible to examine whether the principles governing the interaction between BHT and O_2

are of general significance and might be applied to better understand some health effects of oxidants.

BACKGROUND

In mice BHT produces extensive and uniform death of the Type I alveolar cells. Within 24 h after a single administration of BHT, necrotic Type I cells disintegrate and slough off, leaving large areas of denuded basement membrane. If the animals are left undisturbed, the changes in the alveolar zone are successfully repaired within the next 2 to 6 d. Recovery is accomplished, as in many other forms of acute toxic lung injury, by proliferation of Type II alveolar cells which eventually transform into Type I cells. A normal air/blood barrier is restored ~1 week after BHT, and the lungs of BHT-exposed animals look essentially normal again. Therefore, a single acute episode of toxic lung damage appears to be without any serious long-term consequences (Hirai et al. 1977; Adamson et al. 1977).

It is, however, possible to interfere with tissue recovery. Once Type II alveolar cells begin to divide following BHT-induced lung injury, they appear to become quite susceptible to elevated concentrations of O_2 in the inspired air and may be killed. Oxygen concentrations as low as 50% have been found to have an adverse effect on epithelial cell division in lung. Interestingly, dividing interstitial or dividing capillary endothelial cells appear resistant to the cytotoxic action of O_2. This observation led us to predict that the presence of O_2 following lung injury would compromise reepithelialization of the alveolar zone without, however, interfering with the proliferation of fibroblasts. A possible consequence would be the development of fibrosis (Witschi and Côté 1977a). The experiments summarized below confirmed this hypothesis.

METHODS AND RESULTS

Animals treated with BHT and exposed to O_2 during the phase of epithelial cell proliferation developed extensive and diffuse fibrosis within 1 to 2 weeks. Quantitative determination of lung hydroxyproline showed that exposure to 50% to 80% O_2 alone for up to 6 d did not increase lung collagen. Injection of BHT alone produced only slight fibrosis. However, combined treatment with BHT and O_2 increased total lung collagen 2 to 3 times above controls. The combined effects of BHT and O_2 were synergistic (Haschek and Witschi 1979). Histopathology showed the animals to have developed diffuse interstitial fibrosis with many ultrastructural features common to the human disease known as Hamman-Rich syndrome (Brody 1980). Similar observations were made when epithelial cell proliferation was inhibited by low and nonfibrogenic doses of x-rays (<200 rads) (Haschek et al. 1980). Animals exposed to O_2 or to x-rays once reepithelialization of the alveolar zone was complete showed no development of fibrosis, however (Haschek and Witschi 1979; Haschek et al. 1980).

In subsequent studies, we used lung hydroxyproline as a quantitative end point to further study this interaction between a bloodborne lung toxic agent and inhaled O_2. Several observations were made. Abnormally high levels of total lung collagen persisted for up to 6 mo (possibly longer) after a single episode of exposure to BHT followed by O_2 or x-rays (Witschi et al. 1980). Interestingly, lung morphology appeared to return to practically normal, although lung collagen levels remained almost twice as high as in controls. The influence of persisting high collagen concentrations on lung function remains to be established. Increased hydroxyproline was also found if exposure to 70% O_2, a concentration which in itself does not produce fibrosis, was limited to 48 h only. The maximum fibrogenic response developed when animals were exposed to O_2 immediately after BHT. Delay of O_2 exposure for 48 or even 96 h still produced fibrosis, but its development was much diminished. Significant fibrosis also developed if animals given BHT were exposed for 2 d to O_2 concentrations of >70%, for 3 d to >60%, or for 6 d to >50%; only 40% O_2 had no significant effect. The most frequently used dose of BHT (400 mg/kg) itself produced some accumulation of lung hydroxyproline. If the dose of BHT was lowered to 200 or 100 mg/kg, no fibrosis developed. Yet exposure to 70% O_2 following a nonfibrogenic dose of BHT was accompanied by the development of fibrosis (Witschi et al. 1980b).

DISCUSSION

From these experiments we draw several conclusions. A form of chronic lung damage--interstitial fibrosis--may result from an acute interaction of two toxic agents in the lung. Timing is of crucial importance for the occurrence of this interaction. Fibrosis only develops if O_2, or possibly any other toxic agent, is present during the phase of epithelial cell proliferation following lung injury. Oxygen will not produce fibrosis once reepithelialization is complete. If it develops, however, fibrosis persists for up to 6 mo or longer. The interaction between O_2 and BHT does not occur because one agent directly enhances the action of the other (Williamson et al. 1978). Rather, O_2 adversely affects a cell population that is different from the original BHT target cells and which has been called into action to repair the initial damage.

It is conceivable that this scenario for the interaction of BHT and O_2 in mouse lung reflects a general principle underlying the development of chronic lung damage. Ample experimental evidence documents that many toxic inhalants and numerous bloodborne agents will produce Type I alveolar cell deaths followed by Type II cell proliferation. Several chemicals are also known to interfere with cell proliferation in the lung (Witschi and Cote 1977b). In principle, fibrosis might therefore develop under anv circumstances in which exposure to a first agent causing alveolar cell death is followed by exposure to a second cytotoxic agent, provided a critically ordered sequence of exposure is observed. It does not seem likely that the temporal relationships needed to produce fibrosis (according to our proposed mechanism) always occur. However, if the right conditions are met, the resulting lesion will likely persist. Although single episodes might easily go unnoticed, repeated episodes would eventually result in diffuse and recognizable lung damage.

CONTINUING STUDIES

Attempts have begun to verify our hypothesis with studies of agents other than BHT and O_2. Thus far, we have not been able to produce fibrosis by exposing animals first to 2 ppm ozone (O_3) followed by 70% O_2. There are three possible explanations: (1) our proposed mechanism for pathogenesis of lung fibrosis is not as universal as assumed; (2) the cell kinetics following exposure to O_3 are different from those following exposure to BHT, and our timing of O_2 exposure following O_3 was wrong; or (3) the initial lesion produced by exposure to O_3 was too small and comparable only to lesions produced by low doses of BHT (50 mg/kg), which have not produced fibrosis. The third possibility needs to be studied in more detailed experiments; such studies could well lead to information on no-effect or negligible levels of O_3 exposure. On the other hand, we have found that most animals treated with 400 mg/kg BHT will die if subsequently exposed for 48 h to 1 ppm O_3. The survivors develop extensive fibrosis. Animals with acutely damaged lung are thus more susceptible to O_3. This illustrates that the adverse effects of a common oxidant on a diffusely damaged lung may be much more serious than the effects on a healthy organ. Finally, it will be possible to study the effects of airborne toxic agents on animals in which diffuse interstitial fibrosis has been previously produced by exposure to BHT and O_2. These studies will permit examination of the effects of oxidants in an animal model which mimics a not uncommon form of human lung disease, chronic interstitial fibrosis.

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WORKSHOP COMMENTARY

D. L. Coffin: I gathered from the paper that there seems to be a correlation of this fibrosis with the degree of epithelial reaction. In view of the apparently strong interaction between macrophages and fibroblastic hyperplasia, have you examined the status of the macrophage as an alternate means of mechanism?

H. P. Witschi: That's a very good point. No, we haven't looked very much at the macrophages. One of the reasons is that we were able to see only comparatively few macrophages in the damaged lungs. Dr. Haschek performed the histology and can confirm this.

I do not think, in this particular instance, that macrophages are involved. Rather, I think that O_2 prevents epithelialization, which results in the formation of fibroblasts. There are some experimental studies showing exactly the same thing. Some very convincing results were reported by Terzaghi [Terzaghi, M., P. Nettesheim, and M. L. Williams. 1978. Repopulation of denuded tracheal grafts with normal, preneoplastic and neoplastic epithelial cell populations. Cancer Res., 38:4546-4553]. Denuded tracheal grafts, when implanted, filled up with fibroblasts in no time. Inoculation with epithelial cells of a certain critical number resulted in no fibrosis.

Question: Did you try altering the order in which you gave BHT and O_3, to see if the O_3 set up a system for BHT damage?

H. P. Witschi: No, we have not done this yet.

Question: You mentioned that you thought this lesion might disappear after 6 mo to 1 yr. If it's fibrosis, why would you expect it to disappear?

H. P. Witschi: What persist are increased levels of hydroxyproline, which probably indicate collagen. Interestingly enough, the change in histopathology at 6 mo is not very impressive, although the lung still has a large amount of collagen.

Question: Could this be just a matter of turnover: you have more of a turnover in your damaged lung than in the undamaged lung? If so, it isn't fibrosis. It's just that more turnover results in more residual hydroxyproline.

H. P. Witschi: No, we have increased synthesis and diminished degradation.

Question: So you had total hydroxyproline increase?

H. P. Witschi: Yes.

Comment: I don't quite understand. Let me put it this way: If you were to do, say, connective tissue stains, would that correspond to your hydroxyproline? In other words, you're saying that you don't have increased collagen tissue but that you do have increased hydroxyproline. That's confusing.

H. P. Witschi: It's merely a question of semantics. What is measured after hydrolyzing the lung is hydroxyproline, or an index for collagen. I think ~90% of all lung hydroxyproline is in collagen.

13. OVERVIEW OF CURRENT AND PLANNED RESEARCH BY THE RESPIRATORY UNIT, OAK RIDGE NATIONAL LABORATORY

PART 1. RAT TRACHEAL TRANSPLANT SYSTEM

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INTRODUCTION AND BACKGROUND

One overriding theme of the research effort in the Respiratory Unit at Oak Ridge National Laboratory (ORNL) is the study of the morphogenesis of lung cancer. A major part of this effort is directed towards developing and utilizing experimental animal model systems to identify agents which might enhance tumor induction or progression in the respiratory tract.

Currently, three approaches are used. The first approach is that of inhalation experiments in which animals pretreated with carcinogens are exposed to irritant gases. The tracheal washing technique developed at ORNL by Schreiber et al. (1975) and further developed there by Yarita et al. (1978, represents a second approach. With this system, we plan to precondition the respiratory epithelium before exposure to aerosols. It should be emphasized that this model is particularly amenable to studying any interrelationship between the nutritional state of the animal and susceptibility. Studies with both of these systems are discussed by Dalbey (Chapter 14 of this volume).

A third approach is the rat tracheal transplant system developed in our laboratory by Kendrick et al. (1974). The rat trachea was chosen as a model because of its structural similarity to the human bronchus. This report discusses current and proposed studies with the rat tracheal transplant, most of which fall under the EPA umbrella.

An inherent limitation in systems in which the carcinogen is introduced into the respiratory tract by inhalation or the more common intratracheal injection is the unpredictability of the site of tumor development. It is also impossible to determine either the carcinogen dose or the duration of carcinogen exposure for any given site of the respiratory tract. Both of the model systems developed in our laboratory (i.e., the tracheal washing technique and the tracheal transplant) have the advantage that the target site for the carcinogen as well as for the cocarcinogen is well defined. An accurate dose of the agent(s) can be delivered, the site of tumor development can be predicted (Griesemer et al. 1977; Nettesheim et al. 1977; Pal et al. 1978), and multiple exposures of the same or different agents can be carried out.

CONTROLLED DELIVERY OF THE TEST AGENT(S)

Obviously, delivery of the agent is a key issue in this model system. Ideally, one would like to test very low levels of substances and have a high degree of control over the rate and duration of exposure. The first tumor induction studies were carried out with polycyclic hydrocarbons (PCH) released from beeswax pellets. The rate of PCH release was determined by measuring the material remaining in the pellet at different time intervals following placement in the transplant.

B. Pal of our laboratory has developed an in vitro assay system to pretest agent release rates prior to placement of pellets in transplants. This method consists of shaking the carcinogen pellets in a flask containing fetal calf serum at 37°C. The serum is changed at time intervals and the carcinogen released into the serum is determined by radio assay. As shown in Figure 13-1, the release rates in vitro and in vivo are concentration-dependent (i.e., as the concentration in the pellet diminishes with time, the amount released per time diminishes). The rate of in vivo release from pellets containing 100 µg PCH is faster (100% released in 2 weeks) than that from pellets containing 1000 µg PCH. At low concentrations of PCH, in vivo and in vitro release rates are similar. At high PCH concentrations, in vivo rates are markedly slower than in vitro rates.

These studies prompted exploration of other possibilities for altering and reducing PCH release. The first to be explored was the effect of adding

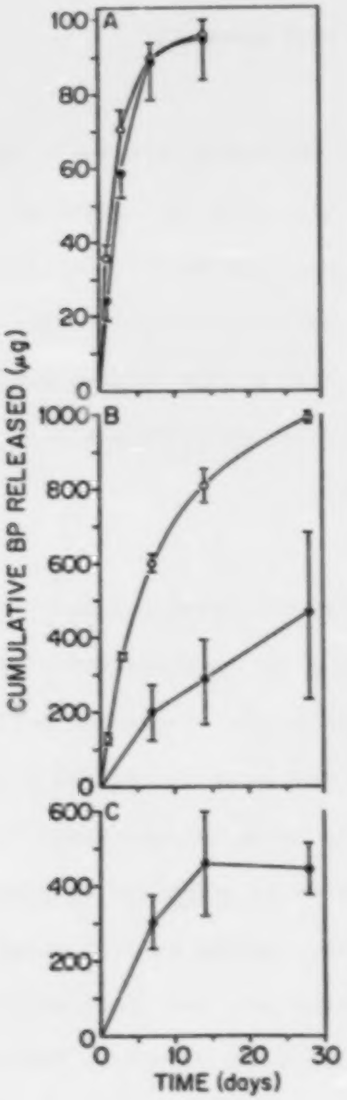

Figure 13-1. Cumulative release of benzopyrene (BP) from beeswax pellets. A, pellets containing 100 μg BP. ○, *in vitro* release; ●, *in vivo* release in tracheal grafts. Each point represents the mean observation from 4 pellets *in vitro* ± S.D. and 6 pellets *in vivo* ± S.D. B, pellets containing 1000 μg BP. ○, *in vitro* release; ●, *in vivo* release in tracheal grafts. Each point represents the mean observation from 3 pellets *in vitro* ± S.D. and 12 pellets *in vivo* ± S.D. C, pellets containing 1000 μg BP implanted s.c. Each point represents the mean observation from 6 pellets ± S.D. From: Pal et al. (1978).

cholesterol or cholesterol laurate to achieve beeswax:cholesterol proportions ranging from 1:1 to 1:9. As seen in Figure 13-2, cholesterol was very effective in retarding BP release. At a beeswax:cholesterol concentration of 1:9, the average amount of BP released *in vitro* within 1 week from pellets containing 100 µg BP was ~13% as compared to ~90% released during the same time from pure beeswax pellets. A similar retardation of BP release was observed in the *in vivo* studies.

Our current effort follows similar lines. Pal is attempting to modify the cholesterol and is pursuing many other kinds of substances, including various polymers and biodegradable materials, as matrices to control the release of agents having a wide range of chemical properties. The goal is to examine the inductive or cocarcinogenic effects of such agents on the respiratory epithelium. Since the model is still under development, we currently use commonly known carcinogens and cocarcinogens. We already have an indication that slow release of a particular carcinogen dose is much more tumorigenic than rapid release. One reason for this, most likely, is a reduction in the initial toxic effect.

INCREASED SPECIFICATION OF END POINTS

The common end point in testing agents for induction and promotability of cancer is actual development of a tumor in animal survival studies. There is a great need for end points that are less costly and time-consuming. Our laboratory is attempting to reduce this end point in time and actually use

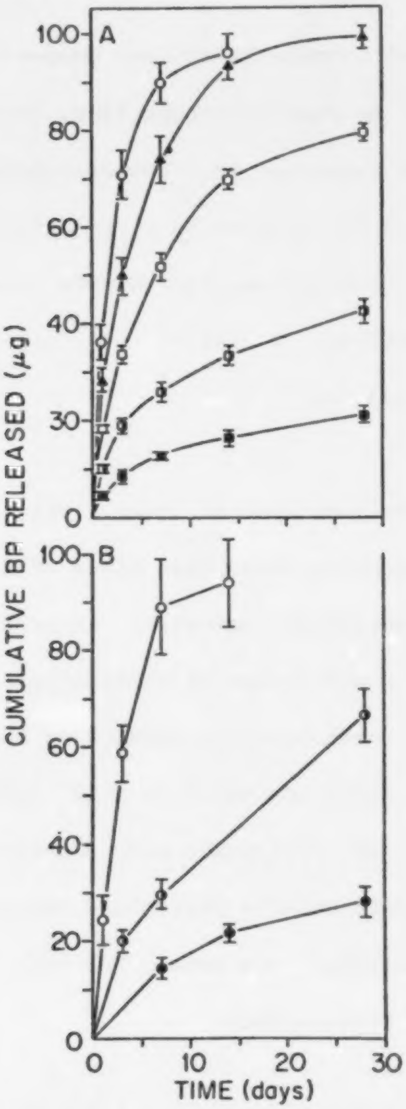

Figure 13-2. Cumulative release of BP from pellets with modified matrix, at 100 μg BP per pellet. A, *in vitro* studies. ○, beeswax pellets; ▲, beeswax:cholesterol laurate pellets at a ratio of 1:9; □, beeswax:cholesterol pellets at a ratio of 1:1; ◨, beeswax: cholesterol pellets at a ratio of 1:3; ■, beeswax:cholesterol pellets at a ratio of 1:9. Each point represents the mean of 2 to 5 observations ± S.D. B, *in vivo* studies in tracheal grafts. ○, beeswax pellets; ◐, beeswax:cholesterol pellets at a ratio of 1:3; ●, beeswax:cholesterol pellets at a ratio of 1:9. Each point represents the mean of 4 to 6 observations ± S.D. From: Pal et al. (1978).

numbers and kinds of lesions identified by ways other than and/or in addition to morphology. The design of these experiments is as follows:

1. Expose transplants to 200 μg dimethylbenzanthracene (DMBA).
2. At set times, sample transplants and cut into 2 x 3 ml explants.
3. Place into organ culture. After 24 h, collect media and prepare the exfoliated cells for cytopathologic diagnosis. Fix explant in one set for pathologic diagnosis. Compare. Place second set of explants into outgrowth culture to determine other markers of cellular alterations.

In studies funded by the National Cancer Institute, we are beginning to follow the fate of cell populations from specific lesions. The properties to be determined are:

1. Rate of epithelial outgrowth
2. Maintenance of primary cultures in suboptimal media (increased *in vitro* growth capacity)
3. Focal morphological changes in the primary cultures
4. Subculturability of the primaries into cell lines
5. Markers of transformation in the cell lines:
    (a) growth in agar
    (b) formation of tumors
6. Correlation of lesion severity as determined morphologically to the time of appearance of markers of transformation

The basic methodology for these studies has been described previously (Marchok et al. 1977, 1978). So far, we have observed the following:

1. A very early marker of transformation is an increased capacity to survive and grow *in vitro*.
2. The brief *in vivo* carcinogen exposure "initiated" some cells, and the processes initiated *in vivo* proceeded *in vitro*, leading to the emergence of some neoplastic cell populations.
3. Since the primary cell cultures and most cell lines were not malignant when first tested, but some became so later, progression of the neoplastic disease must have occurred *in vitro*.
4. There were dose-related effects:
    (a) More primary cultures and cell lines could be established from tracheas exposed to a high (640 µg DMBA) than a low (165 µg DMBA) dose.
    (b) There were differences in the *in vitro* "latency" periods (i.e., the time cells are maintained before becoming oncogenic).
    (c) Tumor induction time (i.e., the time from cell inoculation to development of a palpable tumor) correlated in most cases with *in vitro* latency. Tumor induction times were 9 to 60 d for cell lines with short *in vitro* latency periods and from 100 to 250 d for cell lines with long *in vitro* latencies.
    (d) The combined *in vitro*-*in vivo* tumor latency time was similar to tumor induction times found in the tracheal transplants *in vivo*.

With this experimental approach, we should achieve the following:

1. Direct determination of the fate of specific lesion types in terms of:

   (a) acquisition of the neoplastic state

   (b) tumor latency

2. Identification of other markers (end points) for determining the tumorigenic potential of cells

3. Identification of cell populations at risk in the respiratory system of experimental animals

4. Use of these end points to test the carcinogenic potential of environmental agents on the respiratory system

5. Application of the same criteria to the cytopathology and to biopsies of human tissue

Looking ahead, it should be emphasized that this approach can be utilized regardless of the form of exposure or type of agent(s) tested.

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WORKSHOP COMMENTARY

D. L. Coffin: In the work that showed an interaction between DMBA and asbestos, how was the asbestos applied to the implanted tracheas?

A. C. Marchok: The asbestos was released from gelatin pellets.

D. L. Coffin: And the DMBA was in the beeswax?

A. C. Marchok: Yes. They were exposed sequentially.

D. L. Coffin: Which came first?

A. C. Marchok: The DMBA came first as the initiator; asbestos was evaluated as a promoter.

D. L. Coffin: How long after DMBA was the asbestos administered?

A. C. Marchok: The gelatin pellets containing asbestos were inserted into the tracheas 4 weeks after the start of DMBA exposure.

14. OVERVIEW OF CURRENT AND PLANNED RESEARCH BY THE RESPIRATORY UNIT, OAK RIDGE NATIONAL LABORATORY

PART 2: TRACHEAL WASHING SYSTEM AND OXIDANT INHALATION EXPERIMENTS

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INTRODUCTION

This report provides an overview of current Oak Ridge National Laboratory studies on inhalation of oxidant gases. Three main areas are discussed: (1) irritant gases as cofactors in nitrosamine-induced tumorigenesis, (2) modulation of tumor incidence using the tracheal washing model for tumor induction, and (3) the role of frequency and duration of oxidant gas exposure in pulmonary toxicity.

Since man is rarely exposed to doses of chemical carcinogens large enough to result in tumors by themselves, investigations of factors which could influence tumor incidence are of great practical importance. Such potential enhancement is the focus of the first two studies described below.

IRRITANT GASES AS COFACTORS IN NITROSAMINE-INDUCED TUMORIGENESIS

In this study, subcutaneous administrations of diethylnitrosamine (DEN) were performed to induce tumors in the respiratory tracts of male Syrian golden hamsters. This use of a systemic carcinogen was designed to decrease the variability in tumor response that is apparently inherent with intratracheal instillation of carcinogens.

Hamsters received 10 weekly injections of 0.5 mg DEN. In addition, groups of animals were exposed to either nitrogen dioxide (NO_2) or formaldehyde (HCHO) for life. These gases were chosen as model lower and upper respiratory tract irritants, respectively. The irritant treatment was further subdivided so that irritant exposures began at one of two times in relation to DEN treatment: (1) 48 h prior to each weekly DEN injection, so that the respiratory epithelium was actively proliferating during the time of carcinogen treatment, or (2) 2 weeks after the final DEN injection. Irritant gas exposures were given weekly for the lifetime of each animal. Ancillary data indicated that such weekly exposures would result in epithelial necrosis and proliferation subsequent to each exposure analogous to that occurring after the initial exposure. In other words, the animals would demonstrate no adaptation with weekly exposures.

After each animal's death, the incidence of respiratory tract tumors was evaluated by means of a clearing technique rather than histology. Fixed tissue was stained with Wright's stain and rendered semitransparent by dehydration and eventual transfer into methyl salicylate. Areas of dense cell aggregation (tumors) stained darkly and were readily visible under a dissecting microscrope. Observations of tumors at this subgross level correlated well with subsequent microscopic evaluation, except that several tumors found at the subgross level would not have been observed during routine histological procedures. There were no false positive or negative tumor identifications during the histological evaluation. All observed tumors were adenomas; no invasive tumors were observed. As of this writing, the experimental data are still tentative. We have not yet fully evaluated the incidence of tumors in control animals.

We obtained data on tumor incidence in animals exposed to DEN and either 10 ppm NO_2 or 30 ppm HCHO. The incidence of nasal tumors was low and not influenced by irritant exposures. Most of the observed tumors were in the larynx and trachea, with a smaller percentage occurring in the lung. With the DEN dose used, the percentage of tumor-bearing animals was high, and no differences in percentage of tumor-bearing animals were observed between experimental groups. However, the number of tumors per tumor-bearing animal was significantly increased in the tracheas of animals that were concurrently exposed to HCHO and DEN. Also, there was a significant increase in total respiratory tract tumors in animals concurrently receiving NO_2 exposures and DEN treatment, although there was no significant increase in either the larynx, trachea, or lung individually.

It appeared from these observations that both irritant gases acted to enhance tumor incidence when the carcinogen was administered at a time of epithelial proliferation resulting from irritant gas exposure. No "promoting activity" was observed.

Partly in view of this lack of observed promoting activity, and also in view of the relative paucity of demonstrated promoting activity in the respiratory tracts of whole animals, we entered into the second major area of work: modulation of tumorigenesis using the tracheal washing technique for tumor induction.

TRACHEAL WASHING SYSTEM

Marchok (Chapter 13 of this volume) has discussed the development of the tracheal washing technique at our laboratory. The anesthetized hamster is placed on its back and a small cannula with a double lumen is inserted through the larnyx. A buffered solution containing N-methyl-N-nitrosourea (NMU) is injected via a syringe pump through the outer lumen of the cannula. The solution moves 5 mm down the tracheal epithelium before reaching the elongated tip of the inner tube of the cannula, where suction draws the solution out of the trachea. Thus, by placing the cannula tip at a standard location within the trachea, a particular site of tracheal epithelium can be repeatedly washed with NMU solution.

This model of tumor induction offers several advantages over intratracheal instillation or systemic administration of carcinogen for whole-animal studies. These advantages include: more quantitative delivery of the carcinogen to a specific site of respiratory epithelium; a small, predictable location of tumor development to facilitate histological sampling of both cancerous and precancerous lesions; and induction of carcinomas in addition to noninvasive lesions. We believe the full potential of this system has not yet been realized, and we are currently utilizing it in multifactorial studies. The first is a promotion study in which the tracheal epithelia of NMU-treated animals are also exposed to a known tumor promoter, croton oil. An aerosol of croton oil provides a noninvasive means to repeatedly treat the epithelium. We also plan tumorigenesis studies involving modification of the physiological state of the animal (namely, vitamin A deficiency). The system is amenable to studies with other agents or physiological alterations.

EFFECTS OF INHALED NITROGEN DIOXIDE ON PULMONIC LESIONS IN RATS

A third area of work in our laboratory concerns the role of duration and frequency of exposure to oxidant gases in the severity of resulting pulmonic lesions. Much of this work relates to the biological effects of acute peaks of oxidant concentration. So far, most of these have involved exposure of specific pathogen free (SPF) rats to NO_2.

As observed in other laboratories, we noted a dose-related increase in incorporation of tritiated thymidine into pulmonary DNA after acute NO_2

exposure. The peak incorporation occurred ~24 h after exposure and was linear with the concentration of exposure. The limits of detection were rather high (~10 ppm NO_2 for a 5-h exposure) when the biochemical method was used to determine thymidine incorporation. Also, we observed an increase in phospholipids after single NO_2 exposures, with a peak in pulmonary phospholipids at ~48 h after exposure. The disaturated species of phosphatidylcholine increased preferentially over unsaturated species, indicating that the increase in phospholipids may be in part due to increased biosynthesis.

We conducted additional experiments on the influence of vitamin A deficiency on the response to acute NO_2 exposures, but the data are incomplete at this time. Vitamin A is important in maintenance of the normal epithelium.

We thought it would be of interest to examine further the relative importance of exposure duration with single acute exposures to NO_2. The end points used were thymidine incorporation, amount of phospholipids, and morphology. We compared 1- and 5-h exposures. Unlike the linear increases in thymidine incorporation and amount of phospholipids in relation to NO_2 concentration, the values for these end points appeared to plateau as exposure duration increased. Presumably the lungs were adapting during the exposure. We subsequently found that only one 1- or 5-h exposure to NO_2 was sufficient to adapt the animals to a similar exposure on the following day. That is, after the second exposure the animals failed to respond with similar evidence of cell necrosis or proliferation.

Adaptation to NO_2 therefore appears to occur rapidly. We subsequently investigated the duration of adaptation. Animals were reexposed either 1, 3, or 7 d after adaptation had been initiated, and thymidine incorporation was monitored subsequent to the second exposure. Data were returned demonstrating that adaptation was complete with a 1-d interval, intermittent with a 3-d interval, and nonexistent after 7 d between exposures. We can assume, therefore, that once-a-week exposures would result not in adaptation to NO_2 but in occurrence of the same lung events observed after the initial exposure.

To our knowledge, the emphysematous lesions that have been reported with chronic exposures of rats were observed under daily exposure regimes in which the animals were probably in the adaptive state. We asked whether similar emphysematous lesions could be produced with weekly exposures of nonadapted rats. SPF male Fischer 344 rats were exposed once per week, 5 h/d, to 20 ppm NO_2. This high concentration was used to ensure an observable effect on the lungs. The experiment has not been completed, but some data on pulmonary function are available.

Some animals were killed after 6 and 18 mo of exposure; additional animals remain in a recovery period. Pulmonary function tests were performed immediately before killing the animals; we obtained data on pulmonary compliance, lung volume, pulmonary resistance, nitrogen washout, and flow volume. These data did not indicate any change in pulmonary function following NO_2 exposure. After the pulmonary function tests, lungs from the animals were fixed under constant pressure and paraffin sections prepared. No change in mean linear intercept was observed after 6 mo of exposure; data on the 18-mo exposure are not yet available.

If subsequent data obtained in this study are similar, the negative results may represent a significant finding that the emphysematous lesions previously reported to occur with chronic NO_2 exposure do not occur under conditions of sporadic peak exposures. Our laboratory is currently planning similar work with ozone, but these plans are readily adaptable to the needs of EPA.

15. EARLY STAGES OF RESPIRATORY TRACT CANCER: A REVIEW

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INTRODUCTION

About 90% of all human cancers appear to originate in epithelia. The epithelial linings of several organs, including the respiratory tract and gut, are topologically continuous with the exterior environment. These linings make up the body's first line of defense against the entry of noxious environmental agents. Simply by reason of their spatial location, epithelia may be particularly susceptible to the toxic and carcinogenic effects of environmental agents. Cumulative effects and delayed effects are of particular concern, because their detection by epidemiologic methods may not be possible until decades after the initial exposure has taken place. An increased understanding of delayed effects--particularly of carcinogenicity--would be useful for identifying and controlling suspect agents.

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To define changes that are specifically correlated with carcinogenesis, we need to identify features that distinguish between normal and neoplastic epithelium at the cellular level. For technical reasons having to do with the growth requirements of cultured cells, the features best understood at the cellular level have been studied in fibroblasts and embryonic mesenchymal cells. While these models are far from ideal for studying carcinogensis, they have the advantage of yielding rapid and quantitative data for a number of experimental end points. These systems have been used to define differences in the surface structure of the cells (Rapin and Burger 1974; Porter et al. 1973; Borek and Fenoglio 1976), contact inhibition of growth (Aaronson and Todaro 1968; Holley and Kiernan 1968), anchorage-independence (Jones et al. 1976; Shira et al. 1975), loss of the protein fibronectin from cell surfaces (Hynes 1974; Yamada and Weston 1975), and increased plasminogen activator activity (Ossowski et al. 1973; Unkeless et al. 1973). All of these differences have been described in the last 10 to 12 years.

Until quite recently, many of us who work on carcinogenesis had considered that at least some of the features that discriminate between normal and transformed fibroblasts would also discriminate between normal and neoplastic epithelium. Such features would provide a "tag" for neoplastic cells that could possibly be applied to a practical problem: defining criteria to use in short-term carcinogenesis assays and in early clinical detection of cancer.

Unfortunately, the prospect of directly applying what has been learned from fibroblast models to studies of epithelium now seems less promising. Recent studies have reported no changes in the amounts of fibronectin on cell surfaces for bladder or for salivary or mammary gland epithelium (Wigley and Summerhayes 1979; Yang et al. 1980). No difference in plasminogen activator activity has been found for normal and neoplastic mammary epithelium (Wigley and Summerhayes 1979). Both our studies (Heckman and Olson 1979) and those of McGrath and Medina on the mammary gland (Voyles and McGrath 1976; Butel et al. 1977) have shown little consistent difference in contact inhibition of growth. Our group has also shown the density of surface features to correlate poorly with the tumorigenicity of cell lines from rat tracheal epithelium (Heckman and Olson 1979). Finally, anchorage-independence and lectin-mediated agglutinability, although somewhat more promising than the other criteria, have not been very reliable in discriminating between normal and tumor-derived human cells (Franks 1979). These two markers are currently being studied by many other research groups.

A few years ago, when we began to look for markers to use in studies of progressive changes in the respiratory tract, we considered that many or most of the biochemical differences that had been described for fibroblast transformation models would relate to their differentiated functions and would therefore be specific to mesenchymal cells. Recent reports by other investigators indicate that this may be the case. Based on this assumption, we decided to concentrate our efforts on a few criteria which had been shown to discriminate between normal and neoplastic epithelium. One of these was the accumulation of ether-linked lipids, which had been described for whole tumors by Snyder and Wood (1968) but studied rarely since then. These lipids, particularly the alkyldiacylglycerols, are present only in trace amounts in normal tissues of most organs. A second marker was described by Montesano and Sanford (Montesano et al. 1977) for normal and neoplastic cell lines grown in culture. These investigators compared several lines and found differences in the morphology and cytology of cells in colonies.

THE ETHER LIPID MARKER

Before studying sequential changes in carcinogen-treated respiratory tract tissues, we wanted to confirm that the lipids occur in tumors from the respiratory tract but not in normal tissues. For this, four transplantable rat tumors obtained after benzo(a)pyrene (BaP) treatment were studied. Thin-layer chromatography showed the alkyldiacylglycerols to be present in the squamous cell carcinomas but lacking in the normal lung (Figure 15-1). All four transplantable tumors had high levels of alkyldiacylglycerols. We wanted to determine how soon after carcinogen treatment this marker could be detected in respiratory tract epithelium. To do this, we used a lipid precursor, 1-^{3}H-hexadecanol, specifically incorporated into the ether linkage (Topping et al. 1978), since mass determinations of the lipids would have required large numbers of animals for quantification, particularly in the case of normal epithelium.

Figure 15-1. Thin-layer chromatogram of lipids extracted from normal lungs (Lanes 1-2) and transplantable carcinomas (Lanes 3-6).

TABLE 15-1. 1-^{3}H-HEXADECANOL INCORPORATION INTO TRIGLYCEROLS AND ALKYLDIACYLGLYCEROLS OF TRACHEAL IMPLANT EPITHELIUM (DPM X 10^{-3})

Treatment	Time After Precursor Delivery (d)				
	1	4	7	21	42
untreated control	3.01 ±1.94 SE	0.72 ±0.36 SE	0.50 ±0.06 SE	0.12 ±0.05 SE	0.13 ±0.02 SE
DMBA (4-week exposure)	18.70 ±12.94	7.58 ±6.12	5.99 ±2.84	0.33 ±0.29	0.21 ±0.19
DMBA (4-week exposure, 16-week reversal)	8.34 ±2.86	4.62 ±1.16	2.36 ±1.88	0.20 ±0.13	-

The animal model used for studies of the lipid marker was the tracheal implant (Marchok, Chapter 13 of this volume) after treatment with the potent carcinogen 7,12-dimethylbenz(a)anthracene (DMBA). To review the sequence of morphological changes obtained with the tracheal implant model, after a 4-week exposure to the carcinogen we observe a squamous metaplastic response mixed with atrophic epithelium. By 2 mo after the exposure is terminated, we begin to see focal areas of squamous metaplasia with atypia. Lesions with marked atypia can be found by 4 mo after termination of the exposure.

A recent paper dealing with the morphological changes induced by various polyaromatic hydrocarbons (Lumb and Snyder 1971) demonstrated that the effects of the carcinogenic hydrocarbons DMBA and BaP are slow to be reversed in comparison to those of the noncarcinogenic or weakly carcinogenic hydrocarbons. Although the lesions obtained with the two carcinogens differ in morphology, they are similar in that they persist in the epithelium. Thus, the concept of reversibility appears to be important in this model system.

Table 15-1 shows the levels of tritium incorporated from 1-^{3}H-hexadecanol into the alkyldiacylglycerol and triglycerol fractions at various times after delivery of the precursor to tracheal implants. The amount of label incorporated into the normal implants was minimal and declined relatively rapidly. The effect of a 4-week exposure to DMBA was striking. The average level of labeled alkyldiacylglycerols was 6 times higher than in the normal epithelium, and the turnover of the label appeared to be slower. Precursor incorporation was also studied at 4 mo after termination of carcinogen treatment (i.e., at the time when atypical lesions can first be detected). The level of label incorporated at this time was 2 to 3 times higher than in the normal epithelium, and the turnover again appeared slower.

With the ether lipid marker, then, we succeeded in identifying a biochemical change that resembles previously studied morphological changes in that it is slow to be reversed. The accumulation of ether lipids, particularly the alkyldiacylglycerols, is also somewhat remarkable in that it occurs very early after carcinogen treatment.

We also wanted to understand the biochemical basis of ether lipid accumulation. To study this problem, we cultured normal rat tracheal epithelium in parallel with cells from one of our transplantable tumor lines. In particular, we wanted to know whether the turnover of the ether lipids was impaired in carcinogen-treated epithelium. If this were the case, then absence of the cleavage enzyme might provide a more direct marker for preneoplastic changes. However, when grown *in vitro*, the normal epithelium synthesized levels of alkyl lipids were equivalent to those of the tumor cells. Incorporation in alkyldiacylglycerols of labeled palmitate (the precursor) was similar for the two cell types, as was the rate of turnover (Scott et al. 1979a).

The unexpected similarity of lipids from normal and neoplastic cells grown *in vitro* suggested that alkyl lipid accumulation might be related to glucose levels, which are high in the culture medium and which are known to be elevated in many neoplastic cells. In a second series of experiments, we showed this to be the case. By growing neoplastic cells from two of our transplantable cell lines in media supplemented with varied levels of glucose, we could induce the accumulation of correspondingly high or low levels of the triglycerides and their ether analogues (Table 15-2). The effect of glucose is related to levels of the precursors needed for biosynthesis of the ether lipids. The precursor for the glycerol backbone is dihydroxyacetone phosphate, which is formed as a direct glycolytic intermediate, according to the Embden-Meyerhof-Parnas scheme. It is not clear how synthesis of the other necessary precursors, the long-chain fatty alcohols, might be related to glucose levels. Our evidence indicates that their biosynthesis is regulated by an as yet unidentified product of glycolysis (Scott et al. 1979b). In any case, these results suggest the feasibility of a more direct assay relating to glycolytic metabolism for application to the problem of detecting preneoplastic changes in the respiratory tract.

THE CELL SHAPE MARKER

We undertook experiments similar to previously described studies using liver cells (Montesano et al. 1977) to examine the possibility that morphological features could serve as markers for early stages of cancer in the respiratory tract. The model system was an *in vitro* system comparable to the DMBA tracheal implant model. Two cell lines derived from the tracheal epithelium after 2 weeks of carcinogen exposure (Marchok et al. 1978) were used. Figure 15-2 diagrams the development of oncogenicity in these lines.

TABLE 15-2. VARIATION OF TRIGLYCEROL AND ALKYLDIACYLGLYCEROL CONTENT WITH LEVELS OF GLUCOSE IN CULTURE MEDIA[a]

Cell Line	Glucose Concentration (mM)	Triglycerol Content (μg/mg protein)	Alkyldiacylglycerol Content (μg/mg protein)
B2-1	0	4.8	5.7
	0.2	12.3	14.6
	1.0	61.6	41.8
	5.5	109.6	75.5
BP3-0	0	21.1	6.7
	0.2	22.2	9.5
	1.0	22.8	13.0
	5.5	35.6	34.6

[a] Data from Scott et al. (1979b).

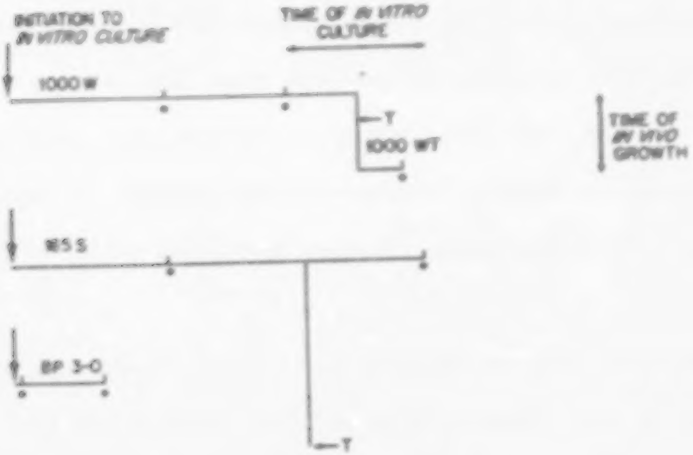
INITIATION TO *IN VITRO* CULTURE
TIME OF *IN VITRO* CULTURE
1000 W
T
1000 WT
TIME OF *IN VIVO* GROWTH
855 S
BP 3-0
T

Figure 15-2. Diagrammatic representation of in vitro derivations and experimental sampling points (•) for three rat tracheal epithelial lines.

The 1000 W line, upon becoming oncogenic, formed tumors in all immune-suppressed hosts tested. As positive and negative controls, a cell line that formed tumors in relatively few sites (165 S) and a highly oncogenic line from a transplantable tumor (BP 3-0) were used.

To ensure that we identified changes linked to the oncogenic status of the line rather than with the amount of time it was carried in *in vitro* culture, a tumor-derived subline of 1000 W was also studied. In this way, we could examine an additional oncogenic 1000 W population that had been maintained in culture for only a brief time.

There were no significant differences in kinetic characteristics of cells in colonies from the early and the tumor-derived 1000 W populations. For example, Figure 15-3 plots labeling index versus the number of cells in the colonies. In colonies from the early passage of the line, the cells tended to be flat and regular. However, in colonies from late passages and from the tumor-derived subline, the cells tended to be more elongated (although many were still regular in shape). A quantitative analysis of the shape criterion confirmed this visual impression (Heckman and Olson 1979).

It is important that we identify the cellular mechanism underlying the changes in cell shape. Particularly if these changes involve an extracellular constituent, they could provide a second marker for *in vivo* changes related to carcinogenesis. One way to approach the problem, in terms of the cellular biology of the model, would be to perturb the cells from nononcogenic

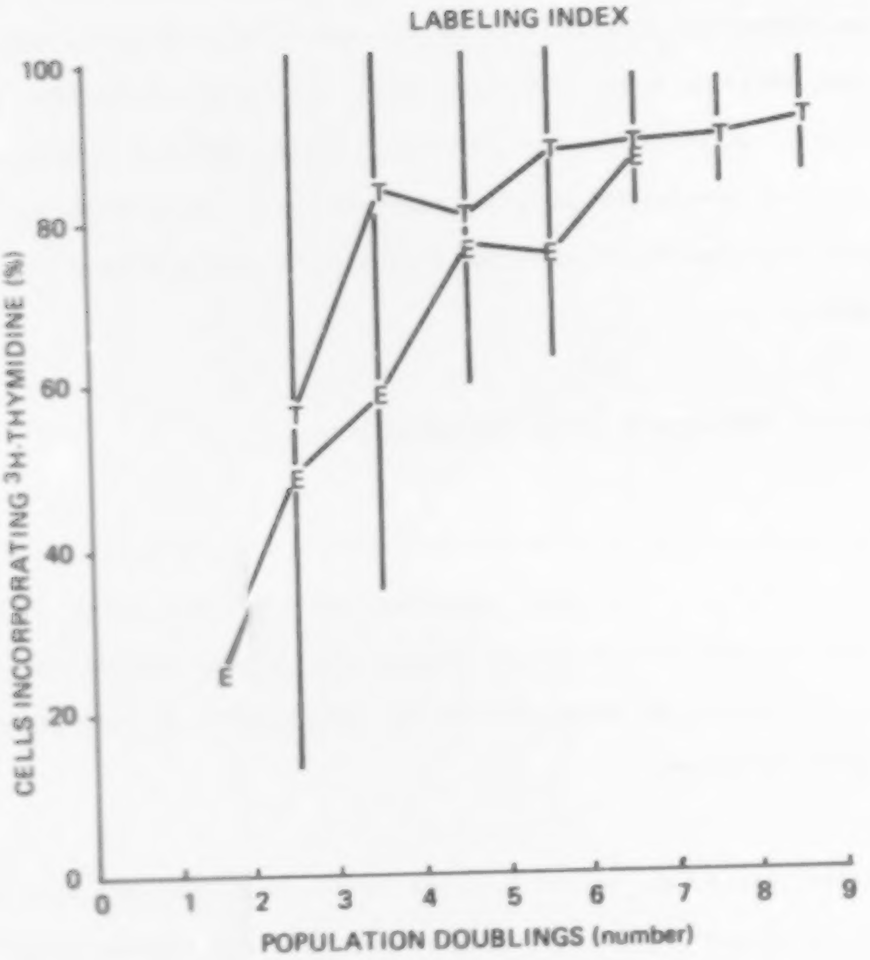

Figure 15-3. Percentage of cells incorporating ^{3}H-thymidine in colonies from the 1000 W (E) and tumor-derived 1000 W (T) lines.

populations and see if they could be induced to mimic the oncogenic populations. To accomplish this obviously requires a more quantitative assay for cell shape, which we developed using a computerized image analysis method. Initial tests demonstrated the method's success in discriminating among cell lines (Olson et al. 1980) (Figure 15-4). In preliminary studies of the 1000 W cell line, we learned that differences in cell shape can be detected in single cells as opposed to colonies. It is also possible to calculate the mean for individual cells in a population and to use that value to estimate the length of time the population has been carried in vitro. While at present it is not clear how this particular end point might be applied to problems of in vivo detection of preneoplastic changes, it remains a promising area for future development.

ANOMALIES IN RESPIRATORY TRACT CARCINOGENESIS

The final section of this review discusses some findings that we consider anomalous in terms of the usual conceptualization of lung cancer etiology. Hopefully, an awareness of these findings will at least prevent us from becoming too complacent about our current understanding of carcinogenesis in the respiratory tract.

Several years ago, we had the good fortune to collaborate with Dr. Walden Dalbey in a chronic inhalation study of the effects of tobacco smoke on the lung. The studies were performed with Fisher 344 rats; thus, the findings may only be indicative of the processes which take place in the lungs of larger

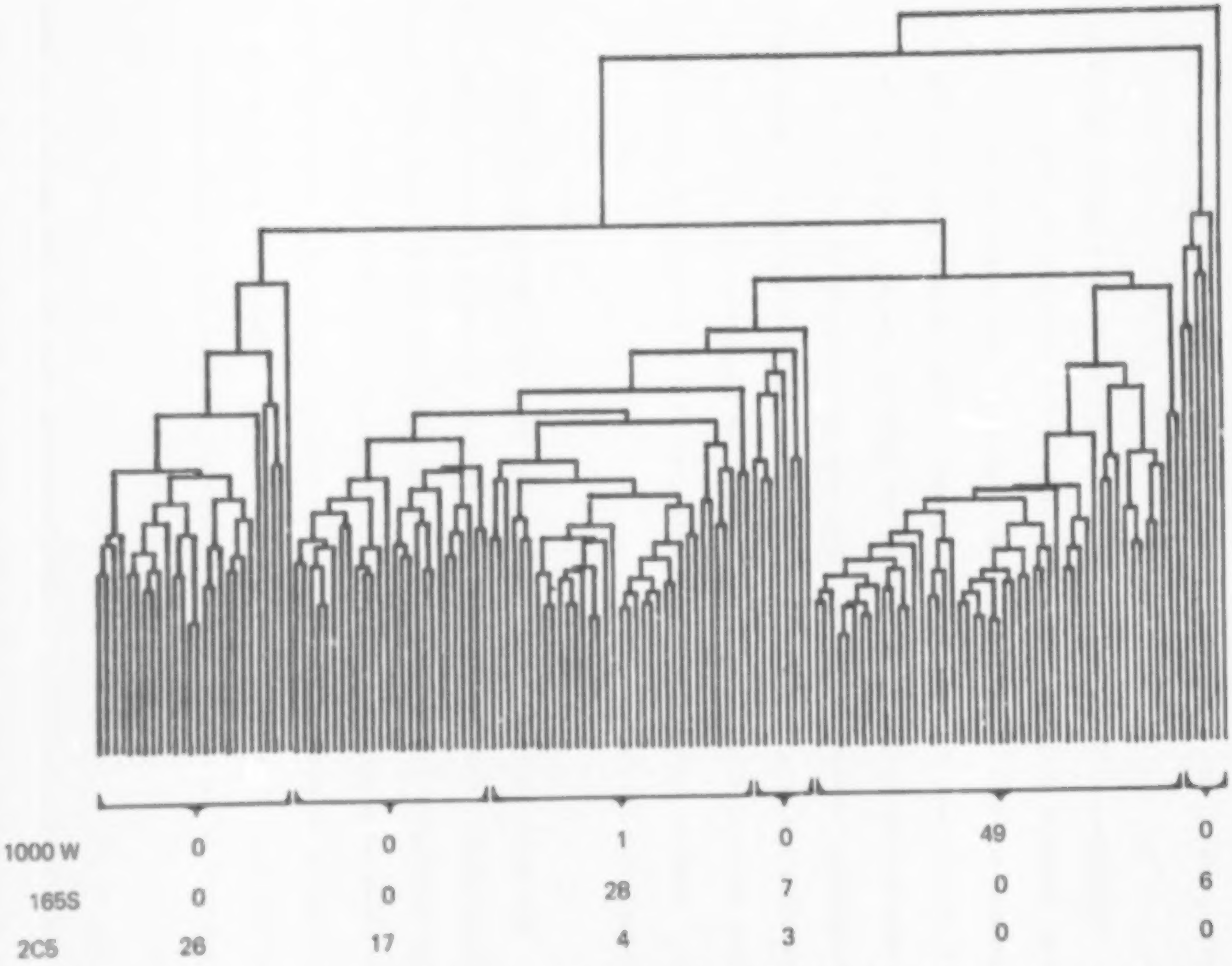

Figure 15-4. Hierarchical cluster analysis of cell shape data input for 50-cell samples from three rat tracheal epithelial cell lines.

animals. However, the results were useful in determining the fate of particulates in the lung in a situation of particulate overloading.

Particulates in the tobacco smoke we studied ranged from 0.1 to 0.6 µm in actual diameter but were accumulated into larger masses within the macrophages. By 3 d after cessation of smoking, nearly all of the particulates were found within macrophages. With chronic exposure, the macrophages were present not only in the alveolar space but also in the interstitial connective tissue. They were particularly common in the connective tissue at the terminal bronchiole and at the pleura. These sites suggested a relationship with the patterns of lymphatic drainage in the rat lung. However, there was nothing more than this circumstantial evidence to implicate the lymphatics.

The major type of lesion found in the lung was typically near the alveolar duct. Most frequently, these lesions developed near sites where the ducts terminated on the pleura. In serial sections, the alveolar duct lesions were often adjacent to a branch of the pulmonary vein which had an accumulation of macrophages in the perivascular adventitia. Because of this juxtaposition, it seems likely that perivascular lesions may contribute to the development of lesions in the parenchyma surrounding the adjacent alveolar duct.

At the cellular level, it seems anomalous that the vast majority of deep lung particulates occur in macrophages while the macrophage does not itself seem to be a target cell for carcinogens in this organ. We know that the macrophage can metabolize BaP, and that it should be able to form reactive metabolites. At one time, cell biologists also thought that the alveolar macrophage did not divide and could not, therefore, be a target for transformation. We now know that these cells are capable of division.

A second apparent anomaly involves the sites in which macrophages accumulate in the lung parenchyma. The most common sites are in the perivascular adventitia, yet the cells of the vasculature do not seem to be target cells, either.

These questions may be resolved when there is better insight into the biological and metabolic characteristics of different cell types in the deep lung. Meanwhile, they should serve to remind us that the field of pulmonary carcinogenesis still poses a number of unresolved questions.

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WORKSHOP COMMENTARY

E. Hu: [Inaudible]

C. A. Heckman: The study I discussed involved measurements only of the alkyldiacylglycerols and ether-linked phospholipids. The gangliosides have not yet been studied in these model systems.

16. CARDIOVASCULAR EFFECTS OF OZONE AND CADMIUM INHALATION IN THE RAT

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INTRODUCTION

At present there is widespread interest and concern about the effects of environmental pollutants upon human health. Much of this concern stems from recent experimental studies showing a variety of environmental pollutants to be carcinogenic. Information on the relationship of pollutants and cardiovascular diseases, however, remains to be determined. Diseases of the cardiovascular system may be grouped into three major categories: hypertension, atherosclerosis (including heart attack and stroke), and chronic heart failure. Together, these three categories represent the major cause of death in North America.

BACKGROUND

The research interests of our group relate to the effects of environmental pollutants (in particular, energy-related pollutants) in cardiovascular disease. In recent studies, we showed that lead and cadmium (energy-related pollutants) induce hypertension and atherosclerosis in the White Carneau pigeon. Similar results were observed in the rat. These observations support epidemiologic studies which show that these elements correlate with hypertension and coronary artery disease (i.e., coronary atherosclerosis). We also obtained preliminary results showing that the chemical carcinogen benzo(a)pyrene induces an increase in the number and size of aortic atherosclerotic plaques in the pigeon. These results show quite clearly that environmental pollutants are involved in cardiovascular disease.

The major routes of intake of environmental pollutants in humans are the gastrointestinal tract and respiratory tract. To obtain the results just described, pigeons and rats were exposed to the pollutants via drinking water (i.e., uptake occurred via the gastrointestinal tract). However, information on the effects of these pollutants in cardiovascular disease following inhalation exposures (i.e., uptake via the respiratory tract) remains to be determined. With the exception of carbon monoxide (an air pollutant associated experimentally with atherosclerosis) we know little about the effects of air pollutants in cardiovascular disease. Nevertheless, oxidants such as ozone (O_3) and the sulfur oxides have been shown to induce reversible electrical changes in the heart as measured by electrocardiographic methods.

In addition, Trams et al. (1972) demonstrated that the activity of monoamine oxidase (MAO) and catechol O methyltransferase (COMT) in the dog brain is significantly reduced after exposure to 1 ppm O_3 for 18 mo. These two enzymes affect blood pressure through their catabolic effect on the neurotransmitter norepinephrine. Thus, a decrease in the activity of these enzymes may increase the half-life of norepinephrine, ultimately provoking an increase in blood pressure.

In previous studies, we exposed rats to 5 ppm Cd via drinking water for 6 mo. After exposure, the animals were anesthetized and the femoral artery pressure and blood norepinephrine measured. Prior to killing the animals, ^3H norepinephrine was injected into the femoral vein, and blood pressure and ^3H norepinephrine levels were measured over a period of 1 h. The results of this study showed both blood pressure and norepinephrine to be significantly increased in the Cd-treated rats. Furthermore, the half-life of ^3H norepinephrine was significantly increased in the Cd-treated animals. As previously noted, the half-life of norepinephrine is in part regulated by MAO and COMT. The half-life is also regulated by cellular uptake (both neuronal and extraneuronal). We previously showed the activity of these two catabolic enzymes to be decreased in the aorta of rats exposed to 5 ppm for 6 mo. Thus the increase in blood pressure observed in the Cd-treated rats may be the result of an increase in the half-life of norepinephrine due to Cd effects on these two catabolic enzymes. However, Cd may also affect the uptake of norepinephrine. In any event, a decrease in the activity of these enzymes can effect a change in blood pressure.

METHODS AND RESULTS

To expand the observations of Trams et al. (1972), we exposed Fischer rats as follows:

(1) O_3, 0.6 ppm, 5 h/d, for 3 d
(blood pressure recorded on day 4)

(2) Cd, 3 mg/m^3, for 1 h
(blood pressure recorded on day 8)

(3) Cd, 3 mg/m^3, for 1 h
4 d later: O_3, 0.6 ppm, 5 h/d, for 3 d
(blood pressure recorded on day 8)

After these exposures, femoral artery pressure, electrocardiographic data, and tissue concentrations of Cd (i.e, aorta, heart, and lungs) were obtained.

Table 16-1 shows the effects of these treatments on the cardiovascular system. Systolic pressure and heart rate were significantly increased in the treated animals. However, diastolic pressure and mean pressure were not significantly changed. The relation of maximal velocity of pressure increase in the femoral artery in the isometric phase of contraction (d_{PP}/dt) was significantly increased by Cd but not by O_3. Note, also, that the effects of O_3 and Cd were not additive with respect to the changes in systolic pressure, heart rate, and d_{PP}/dt. In fact, the changes observed in the Cd plus O_3 treated rats more closely resembled those observed in the Cd-treated animals.

TABLE 16-1. EFFECTS OF CADMIUM AND OZONE INHALATION ON THE CARDIOVASCULAR SYSTEM IN THE FISCHER RAT

Group[a]	Systolic Pressure (mm Hg)[b]	Diastolic Pressure (mm Hg)[b]	Mean Arterial Pressure (mm Hg)[b]	Heart Rate (beats/min)[b]	d_{FP}/dt[b]
Cd (5)	116 ± 22	95 ± 25	102 ± 20	370 ± 80	2500 ± 400
Cd (5)	139 ± 10[c]	81 ± 3	100 ± 7	456 ± 30[c]	3300 ± 500[c]
O_3 (5)	143 ± 9[c]	86 ± 4	105 ± 3	414 ± 55[d]	2700 ± 200
Cd + O_3 (5)	139 ± 10[c]	82 ± 2	100 ± 5	456 ± 40[c]	3050 ± 700[c]

[a] Number of animals per group is given in parentheses.

[b] Mean ± S.E.M.

[c] $p < 0.01$.

[d] $p < 0.05$.

The significant increase in the QRS interval and in the amplitude of the R wave (Table 16-2) suggests that these treatments may have affected the permeability of the myocardial membranes or the metabolism of the neurotransmitter norepinephrine. The increase in heart rate would support the latter suggestion. However, an effect on the permeability cannot be ruled out.

Table 16-3 shows the tissue distribution of Cd. As might be expected, lung Cd was significantly increased (by a factor of 5) following Cd exposure. Aortic Cd increased significantly, while the concentration of Cd in the heart did not significantly change following Cd treatment. In the Cd plus O_3 treated rats, Cd was decreased in the lung and increased in the aorta (as compared to rats treated only with Cd, suggesting that O_3 may increase Cd clearance from the lung.

These results demonstrate that Cd and O_3 have similar effects on the cardiovascular system. The results also suggest that the cardiovascular effects may be exerted through an effect on norepinephrine metabolism. With respect to effects on the cardiovascular system, no interactions between O_3 and Cd were evident.

TABLE 16-2. EFFECTS OF CADMIUM AND OZONE INHALATION ON THE ELECTRICAL PROPERTIES OF THE HEART IN THE FISCHER RAT

Group[a]	Heart Rate (beats/min)[b]	PR Interval (s)[b]	QRS Interval (s)[b]	Amplitude of P Wave (mV)[b]	Amplitude of R Wave (mV)[b]
Control (5)	370 ± 80	0.047 ± 0.003	0.021 ± 0.009	0.0060 ± 0.0004	0.054 ± 0.009
Cd (5)	456 ± 30[c]	0.047 ± 0.007	0.034 ± 0.003[d]	0.0034 ± 0.0006[c]	0.070 ± 0.006[c]
O_3 (5)	414 ± 85[d]	0.047 ± 0.001	0.038 ± 0.0009[c]	0.0076 ± 0.001[c]	0.067 ± 0.004[d]
Cd + O_3 (5)	456 ± 40[c]	0.044 ± 0.001	0.039 ± 0.005[c]	0.0084 ± 0.008[c]	0.069 ± 0.006[c]

[a] Number of animals per group is given in parentheses.

[b] Mean ± S.E.M.

[c] $p < 0.01$.

[d] $p < 0.05$.

TABLE 16-3. TISSUE DISTRIBUTION OF CADMIUM IN THE FISCHER RAT FOLLOWING CADMIUM AND OZONE INHALATION

	Level of Cd in Tissue (µg/g dry weight)^{b}		
Group^{a}	Aorta	Heart	Lung
Control (5)	0.44 ± 0.09	0.24 ± 0.04	1.9 ± 0.7
Cd (5)	0.82 ± 0.07^{c}	0.31 ± 0.03	11.3 ± 1.4^{d}
O_3 (5)	0.50 ± 0.008	0.29 ± 0.07	2.4 ± 0.8
Cd + O_3 (5)	1.29 ± 0.13^{d}	0.27 ± 0.05	10.3 ± 2.3^{d}

[a] Number of animals per group is given in parentheses.

[b] Mean ± S.E.M.

[c] $p < 0.01$.

[d] $p < 0.001$.

RECOMMENDATIONS FOR FURTHER RESEARCH

Based on these preliminary studies, our group offers the following recommendations for future research:

(1) Determine if O_3 has a dose-dependent effect on the cardiovascular system

(2) Determine the effects of O_3 on norepinephrine metabolism

(3) Determine the effects of other oxidants on the cardiovascular system

(4) Determine if oxidants interact with other air pollutants in effecting a change in the cardiovascular system

(5) Determine the effects of various air pollutants in animals with cardiovascular disease

WORKSHOP COMMENTARY

J. L. Whittenberger: In measuring the effects of O_3 exposure on heart rate and blood pressure, did you measure pulmonary ventilation? I wonder whether hypoventilation might have played a role in producing the cardiovascular effects.

N. W. Revis: The effects may very well be due to hypoventilation. They may as well be attributable to the change in the pulmonary system rather than the secondary effect; I'm not sure and cannot give a specific answer to your question. The results suggest to me that the effect is not simply an effect on the sympathetic nervous system required to stimulate the heart rate, thus affecting systolic pressure.

J. L. Whittenberger: I was talking only about the level of ventilation, not about the damage to the lungs. What dose of O_3 was administered?

W. E. Dalbey: The dose was 0.6 ppm for 5 h for 3 d in a row. We measured pulmonary resistance for totally other purposes during the O_3 exposure, but the present animals were not examined until the following day. We didn't do the measurements until ~24 h after the last day of exposure.

17. EFFECTS OF NITROGEN DIOXIDE AND 3-METHYLFURAN INHALATION ON THE SMALL AIRWAYS IN THE MOUSE

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INTRODUCTION

Small airways, i.e., the smallest bronchi and bronchioles, are one of the most vulnerable regions in the lung for damage by many inhaled irritants. Small airways are a major site of airflow obstruction in chronic bronchitis, bronchiectasis, and, probably to a large extent, emphysema (Macklem et al. 1971; Hogg et al. 1968). Despite this fact, few details are available concerning the response of the epithelial components to injury in this region; most attention has centered on the alveolar zone of the lung.

The cell population of the small airways consists of the ciliated cells (responsible for the movement of small particles up the mucociliary escalator) and the nonciliated or Clara cells (the progenitors of the ciliated cells). Although the exact function of the Clara cells is still under investigation, they are secretory cells and are rich in mixed function oxidases. The Clara cells are thus capable of activating drugs and other compounds to highly reactive and toxic metabolites, and of inactivating others. Small numbers of goblet cells, responsible for mucous secretion, are also present in the small bronchi.

The ciliated cells of the terminal airways are readily damaged by the oxidants ozone (O_3) and nitrogen dioxide (NO_2). Following a single exposure to either O_3 or NO_2, the airway epithelium is rapidly repaired due to proliferation of nonciliated bronchiolar epithelial cells which subsequently differentiate into ciliated cells. Since O_3 and NO_2 are the major oxidants present during peak traffic hours, their potential contribution to small airway disease in man must be considered.

Exposure of man to very high NO_2 concentrations has been reported to result in bronchiolitis obliterans. Bronchiolitis obliterans is a particularly severe form of small airway disease in response to local injury of the wall. Initially, cellular granulation tissue more or less fills the bronchiolar lumen. This tissue then undergoes organization and takes a polypoid form, the final fibrosis extending to the musculoelastic and peribronchial regions. Partial to complete obstruction of the bronchiolar lumen occurs. Bronchiolitis obliterans has also been reported to follow exposure to sulfuric acid, ammonia, and war gases. In a study of O_3 exposure in rats (Last et al. 1979), a focal polypoid thickening of alveolar duct walls was observed. This lesion was partially reversible on removal from O_3.

Nonciliated bronchiolar epithelial or Clara cells were recently shown to be the target cells for several toxic furans, including 4-ipomeanol and 3-methylfuran (3MF) (Boyd 1980). 3MF has been identified in city smog and is believed to be formed by photoxidation from naturally occurring terpenes which originate from deciduous trees. The Clara cell activates these compounds to their toxic metabolites, causing cell death. With a single exposure to a low or moderate dose of 3MF, complete regeneration of the bronchiolar epithelium takes place within a few days; following a high dose, regeneration is not fully complete even after 3 weeks (Haschek, unpublished observations).

The study described below was designed to examine whether damage to the progenitor cell--the nonciliated cell--would interfere with successful recovery of the injured bronchiolar epithelium.

METHODS

NO_2 was chosen to produce necrosis of the ciliated bronchiolar cells; a single exposure to 20 ppm for 24 h was used. Inhalation of 3MF at a dose of 2.5 µl/liter for 1 h was employed to damage the nonciliated bronchiolar cells. Young adult male Balb/C mice were divided into five groups and treated as follows:

Group	Treatment		
	Day 1	Day 2	Day 3
A	NO_2	-	-
B	NO_2	3MF	-
C	NO_2	3MF	3MF
D	-	3MF	-
E	-	3MF	3MF

Half the animals in each group were killed on the 6th day; the other half were killed on the 10th day. Animals were killed by cervical dislocation, and lungs were fixed in situ by intratracheal instillation of 10% buffered formalin. Lung tissue was processed, embedded in paraffin, sectioned at 3-4 µm, and stained with hematoxylin and eosin.

RESULTS

A large proportion of the mice exposed consecutively to 3MF died; therefore, groups C and E will not be discussed further. Lungs from animals exposed to NO_2 alone (group A) showed minimal changes consisting of mild hypercellularity around bronchioles and alveolar ducts at 5 d. By 10 d, the lungs appeared virtually normal.

Animals exposed to 3MF only (group D) had marked loss of nonciliated bronchiolar cells. In small bronchioles, segmental areas of denudation were interspersed with squamous and nonciliated cuboidal cells. In larger bronchioles, normal ciliated cuboidal and small groups of columnar nonciliated cells were present. After 10 d, regeneration was evident: bronchioles were lined by varying numbers of undifferentiated cuboidal-to-columnar cells, as well as some normal ciliated and nonciliated cells.

Lung changes in animals exposed to NO_2 and subsequently to 3MF (group B) were similar to those seen following exposure to 3MF alone, with the exception that only small numbers of normal ciliated cells remained in the large bronchioles. Scattered small fibrocellular polypoid masses, covered by squamous to cuboidal epithelium, as well as sessile fibrous thickenings of the bronchiolar wall, protruded into the lumen. After 10 d, regeneration was evident, with many undifferentiated nonciliated cuboidal cells, some dome-shaped Clara cells, and small numbers of ciliated cells. Only a few polypoid lesions remained.

DISCUSSION

This study demonstrated an interaction between NO_2 and 3MF in the small airways. While the ciliated cell injury produced by NO_2 was virtually repaired by 5 d, and the effect of 3MF was primarily on the nonciliated bronchiolar cells, the combined effect of these two agents greatly retarded regeneration of ciliated cells and produced focal thickening and polypoid lesions in the bronchiolar wall. The retardation of ciliated cell regeneration is attributed to damage of the nonciliated progenitor cells by 3MF. Sustained loss of ciliated cells might severely impair the defense mechanisms of the ciliary escalator.

The pathogenesis of the bronchiolar polyps can only be conjectured. The polyps seen in this study were similar in both appearance and reversibility to those produced in the rat by exposure to O_3 (Last et al. 1979). The polyps produced by O_3 were located in the alveolar ducts, whereas in the present study they were located in the bronchioles. The difference in location can be explained by the difference in principal site of injury between these agents. Since simple epithelial damage is usually repaired without the formation of such polyps, one may ask whether damage to underlying elements (such as basement membrane or subepithelial tissue) may play a role in this phenomenon. In bronchiolitis obliterans, where granulation tissue polyps partially or completely occlude the bronchiolar lumen, damage to the bronchiolar wall is implicated. We produced a lesion similar to that of bronchiolitis obliterans by intratracheal instillation of a coal liquefaction distillate in the rat (Haschek et al. 1981). Extensive necrosis of the airways epithelium allowed leakage of the distillate (visualized by fluorescence microscopy) through the mucosa into the underlying tissue and even, in areas, into the surrounding parenchyma. Proliferation of the subepithelial connective tissue resulted in polypoid lesions in the airways and alveolar ducts. These lesions became somewhat condensed with time but were still present at 60 d. Whether such lesions persist or not may depend on the severity of initial damage, the degree of the proliferative response, or, as in the case of the distillate, on the persistance of the agent within the lesion.

In conclusion, despite our recognition of small airway disease as the underlying cause of airflow obstruction in chronic obstructive lung disease and our knowledge that small airways may be severely affected by inhaled oxidants (e.g., NO_2 and O_3) as well as naturally occurring toxins (e.g., 3MF), little is known about the pathogenesis of small airway disease. The response of the small airways to various types and severities of injury, as well as to various combinations of naturally occurring irritants and toxicants, needs to be examined in detail before an understanding of small airway disease can be reached.

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WORKSHOP COMMENTARY

Comment: Putting fairly toxic materials into the lung either by inhalation or by intratracheal administration will induce more than a mere destruction of epithelial cells. The subsequent organization of the inflammatory process (which can be reversible) might be the source of such a fibroblast proliferation. This would agree with Dr. Witschi's observations on the fibrosis that follows inhibition of epithelial cell regeneration. Hitting small airways so heavily as to induce such severe pathologic changes should not be interpreted as unique to the agent, but rather to the degree of damage that is produced.

W. M. Haschek: I agree. I think the airways have very limited ways in which they can respond, and that the response is due to the degree of damage and not to the agent.

R. P. Sherwin: There's quite a distinction between the polyps of your inhaled agents and the ones produced by the distillate. One was edema, raising the bronchiolar surface. This is why we should be very careful to distinguish between cellular damage with edema and spindly epithelial cells as opposed to true proliferative fibro-collagenous depositions. In humans there are many counterparts to these so-called "benign fibrous polyps."

W. M. Haschek: I agree with most of your comments. However, it wasn't simply edema that raised the surface of the bronchiole. There was cell proliferation--proliferation of the underlying fibrous tissue--within these polyps.

R. P. Sherwin: I didn't mean that it was simply edema, but that any fibrous proliferation may be a small part of the lesion. There is a normal turnover of interstitial cells, and a temporary increase in turnover following injury may be occurring. While you may be right about the fibrosis, should it disappear, it would be contrary to the usual human fibrosis, where destruction and fibrous tissue replacement are characteristically persistent. The key question is what absolute amount of collagen is found. We must bear in mind that several studies of lung fibrosis have failed to show increased concentrations of collagen. There is a controversy about this--

H. P. Witschi: There is no controversy; that has been resolved. If you have a fibrotic lung and take an aliquot only for analysis, not only total collagen but also lung weight has increased. If you divide collagen by weight, the "specific activity of collagen," so to speak, stays the same. If you measure collagen per total lung, the value increases.

R. P. Sherwin: The last statement pertaining to total collagen content of the lung is the critical point. Are we dealing with an absolute or relative increase of collagen? A very simple and real explanation for some if not most of the collagen increase in human lungs with fibrosis is the phenomenon known as condensation fibrosis. When sensitive epithelium is lost, the more resistant interstitial tissues of the alveolar walls become stacked; i.e., the alveolar walls collapse and fuse. Also, alveoli are completely lost and in part are replaced by bronchioles with normally thicker walls. The key question, again, is how to distinguish between relative and absolute collagen increases. Is there more absolute collagen?

H. P. Witschi: Yes. If you calculate collagen per total lung, there is more. However, if you just take a few grams of lung, determine collagen, and calculate collagen for every gram of lung, you do not find more.

R. P. Sherwin: Ron Crystal of the National Heart, Lung and Blood Institute (Pulmonary Branch) remarked on this interesting paradox in his own studies (i.e., a discrepancy between the histological and biochemical data). At a meeting we jointly attended, I proposed to him that condensation fibrosis would be a plausible explanation. At any rate, there is a problem that has not been completely resolved.

Comment: Ron Crystal's problem with increased lung collagen is supposedly in idiopathic pulmonary fibrosis. It may or may not be unique to the patients he's investigated. Some investigators do see an increase in whole lung collagen. Whole lung collagen, as I understand it, is measured by analysis of hydroxyproline, directed to the total lung. I think this is what Dr. Witschi mentioned. If there is an increase in whole lung hydroxyproline, there is (in all probability) some form of fibrosis. Some forms are visible as scars; I think some are visible with special collagen stains. What Dr. Witschi described is diffuse or interstitial fibrosis. Some of the collagen is not visible but still apparent as an increase in whole lung collagen.

Question: How much human lung disease is idiopathic fibrosis?

Comment: Quite a lot of it.

Comment: It is a common problem. There are other kinds of fibrosis, however, which constitute a very significant portion of the disease.

W. M. Haschek: The reversibility of lung fibrosis was mentioned. This is a very important question. Human fibrosis does not appear to be reversible. However, human cases of fibrosis frequently are not detected until a late, chronic stage. With bleomycin there is initially fibrosis, but after a year there is no detectable increase in lung collagen. Therefore, there is some indication that very early so-called "fibrosis" may be reversible. Certainly this aspect needs investigation.

[Note: Pickrell (Chapter 24 of this volume) reports a finding of increased collagen but no histological evidence of fibrosis, and thinning of the alveolar walls.]

18. OVERVIEW OF RESEARCH AT LAWRENCE BERKELEY LABORATORY

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INTRODUCTION

This report summarizes two oxidants research projects that were conducted at Lawrence Berkeley Laboratory under EPA sponsorship. The first project examined cocarcinogenic effects of nitrogen dioxide and sulfur dioxide in mice (Dr. White, principal investigator). The second examined the effects of low-level ozone exposure on serum lipoproteins in guinea pigs (Drs. Lindgren and Shu, principal investigators).

COCARCINOGENIC EFFECTS OF NITROGEN DIOXIDE AND SULFUR DIOXIDE IN THE MOUSE

This project was designed to investigate whether the toxic gases nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) are tumorigenic by themselves, whether they promote tumorigenesis, or whether they are in fact cocarcinogenic.

Selection of Model System and Test Gases

For our model system we employed the lung adenoma initiated by the drug urethan. Urethan is carcinogenic in the mouse lung without promotion but can also be promoted by a number of suitable agents. The advantage of the urethan system is that the incidence of lung tumors is lineally dependent upon dose. Also, urethan's action is very quick (95% is excreted in ~8 h), so the time of exposure is very precise. Finally, the tumors can very easily be counted by gross methods quite similar to those employed at Oak Ridge National Laboratory, where the whole lung is dissected. The nodules in the lung are counted using a dissecting microscope.

We chose to test NO_2 because it has been shown to influence a number of Type II alveolar epithelial cells in the mouse lung. The fact that the majority of source cells for mouse lung adenomas appear to be Type II alveolar epithelial cells suggested the possibility of a preconditioning phenomenon that would increase the number of lung adenomas from urethan or other appropriate carcinogens. We chose to also test SO_2 because there are at least two reports in the literature indicating that SO_2 is a promoting cocarcinogen (in other words, that SO_2 exposure after an initiating event with an incomplete carcinogen will increase the yield of tumors).

Experimental Protocol

Randomized CF1 mice were treated with the test gases either before or after exposure to urethan. Exposures to NO_2 or SO_2 were performed in standard chambers, 24 h/d, except for a very brief break for removal of feces and change of food. Chamber gas levels were continuously monitored and recorded.

The NO_2 exposures were 20 ppm for 2 d, 10 ppm for 4 d, and 5 ppm for 8 d. Air controls were in the chamber for 6 d. The SO_2 exposures were: 40 ppm for 3 d, 20 ppm for 6 d, and 10 ppm for 12 d. Controls again consisted of urethan alone and gas alone. All animals survived these exposure regimens.

The mice were serially sacrificed at predetermined times, and the necessary fixing and counting of tumors were completed. Part of each animal's lung was histologically prepared for the counting of Type II cells.

The resulting multivariate data were analyzed using the Cox Relative Risk Model for dose-dependent or time-dependent events that are quantal in nature. It is extremely important, in multivariate studies, to have independent controls for each of the treatment groups, to avoid a multiple comparison with a single control group. As noted, our protocol included such controls.

Results: Nitrogen Dioxide

The level of adenoma production by NO_2 alone (without urethan) was essentially zero (~0.02 adenoma per mouse over the very large sample of control animals). Thus NO_2 alone had no carcinogenic activity.

Analysis of the data also indicated no difference between the effects of NO_2 given before treatment with urethan and given after treatment (same exposure levels). In other words, NO_2 before urethan was the same as NO_2 after urethan. There was no significant difference even when the data were pooled. NO_2 demonstrated neither promotion nor anticarcinogenic effects. Variation in control values over replications reinforced this assessment: the range of control values in the three replications was just about the range of the data.

At present, Type II cell counts are not complete for this data set.

Results: Sulfur Dioxide

When given without urethan, SO_2 (like NO_2) demonstrated no effect on the production of adenomas.

In contrast to NO_2, however, there were notable differences in the results of SO_2 exposure before and after administration of urethan. All concentrations of SO_2 depressed the incidence of tumors when given after urethan and increased the incidence of tumors when given before urethan. The tumor incidence in animals exposed to 20 ppm and 40 ppm was essentially identical, while the effects of 10 ppm differed markedly for "before" and "after."

When SO_2 was given before urethan, significant increases in the incidence of tumors were seen at 20 ppm and 40 ppm SO_2. Such increases in the number of tumors produced do not qualify as "promotion," since initiation had not taken place. The effect may be due to some change in the cellular population which is ultimately exposed to urethan and in which the tumors are produced. At the other exposure level (10 ppm), there was an insignificant difference from control values.

When SO_2 was given after urethan (i.e., the standard initiation and promotion model), we found essentially the opposite effect. For all levels of SO_2, there was a significantly suppressed incidence of urethan-produced adenomas in the lung. Such an effect is termed "suppression" (or "anticarcinogenic effect").

Type II cells counts are complete for this data set. There was no significant difference from control values throughout the exposure regimen.

Ongoing and Planned Studies

Due to the apparent lack of effect by NO_2, we have discontinued all work with that gas. An ongoing study with SO_2 ties in to the previous work by employing one exposure level--20 ppm for 6 d--for which data were collected in the first experiment. The next group of animals will be exposed to 6.7 ppm for 18 d, and we will again examine both pre- and post-exposure effects.

Depending on the outcome of the 6.7 ppm study, we may proceed to a carcinogen other than urethan. (Urethan, a carbamate, is a highly artificial carcinogen that is not known to be carcinogenic in man.) Most likely, we will employ benzo(a)pyrene (or another carcinogen), lower the SO_2 exposure level, and increase the exposure time. We do believe that we are "in the right ballpark" in terms of exposure criteria.

EFFECTS OF OZONE ON SERUM LIPOPROTEIN CONCENTRATIONS IN THE GUINEA PIG

Due to the oxidative nature of ozone (O_3), many interrelated aspects of lung metabolism involving lipids might easily be influenced by this very reactive substance. For example, the Type II cells that proliferate after O_3 exposure use exogenous lipids during surfactant synthesis. Prostaglandins, which require essential fatty acids for synthesis, also seem to be involved in O_3 toxicity. Protection against O_3-induced peroxidation of unsaturated fatty acids has been reported by Donovan and Menzel. In addition, Brown and others have shown that human deaths due to cardiovascular disease are decreased during periods of lower urban pollution. This accumulation of experimental and epidemiologic evidence led us to consider whether the lipoproteins (which have a very significant influence on atherosclerosis and cardiovascular disease) are in fact changed by exposure to O_3.

As mentioned earlier, Drs. Lindgren and Shu served as principal investigators for this study. Their complete report, "Serum Lipid and Lipid Protein Concentrations Following Exposure to Ozone," has been accepted for publication in the Journal of Environmental Pathology and Toxicology.

Experimental Protocol

Hartley guinea pigs were exposed to O_3 for 22 d. The nominal exposure level was 1 ppm; in retrospect, we believe the actual concentration to have been closer to 0.8 or 0.85 ppm. As in the NO_2/SO_2 study, standard exposure chambers were employed. We monitored all of the usual toxicological indicators, such as body weight, growth, food consumption, and mortality. In addition, serum lipoprotein values were obtained at the beginning of the study, at the end of the study, and for 30 d after exposure.

Results: Toxicological Indicators

As expected, we observed a very marked sex-dependent effect of O_3 exposure. There were 2 deaths in the male group but none in the female group.

Other investigators (in our laboratory and elsewhere) have noted that the female is much more resistant than the male for any end point in O_3 exposure.

As predicted from our rat studies, there was a sharp decrease in food intake. This decrease in food intake was accompanied, surprisingly, by no change in body weight. Body weight was maintained at the control levels despite a 35% drop in food intake. Retrospectively, in light of other 1 ppm exposure studies showing (1) a sharp drop in thyroid hormone levels and thyroid function and (2) sharp reductions in growth hormone and thyrotropin from the pituitary (Clemens and Garcia), it's not surprising that the metabolic rate of the animals was sharply reduced.

Results: Lipoprotein Levels

We examined high-density lipoproteins, low-density lipoproteins, very low density lipoproteins, triglycerides, and cholesterol.

There was a very large increase in the cholesterol levels of the exposed animals as compared with normal controls. In males, this increase was 200%; in females, 100%. Comparisons with pair-fed controls (to remove any dietary effect) revealed smaller increases in cholesterol: 50% in males and 30% in females.

Triglycerides and high-density lipoproteins were essentially unaffected compared to either pair-fed or normal controls. There was, in fact, no difference between pair-fed and normal animals for these parameters. On the other hand, low-density lipoproteins were increased by 100% (males) and 50% (females) in comparison to pair-fed controls. The very low density lipoproteins were increased by 100% (males) in comparison to pair-fed controls.

The probability values for these observations are well below 0.001, so the findings are highly significant.

Thirty days after the exposure was terminated, the changes in lipoproteins and cholesterol were still evident. Histologically, the lungs had returned to normal.

Ongoing and Planned Studies

We recently conducted a few more studies in surviving animals from this study. At 8 to 10 mo following cessation of exposure, the animals are returning to normal and probably are not different from normal. We have no intervening values. Depending on EPA interest, we will presumably repeat this study at 0.2 ppm.

19. OVERVIEW OF RESEARCH AT THE UNIVERSITY OF CALIFORNIA - DAVIS

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INTRODUCTION

This report provides a brief overview of oxidants research within the Laboratory for Energy Related Health Research at the University of California - Davis. Our interpretation of the concepts of scientific relevance and merit is presented. Elsewhere in this volume, Dungworth (Chapter 20) discusses our work with ozone, and Raabe (Chapter 21) describes our research on rodent and primate reparative and adaptative mechanisms to coal fly ash and sulfur compounds.

Our research is not performed in isolation: we have an interdisciplinary program that bridges several of the colleges and falls under the aegis of various organized research units as well as individual scientists. Our sister research unit, the California Primate Research Center, is involved in major research efforts on the effects of particulate and gaseous pollutants in animal systems.

APPROACH TO RESEARCH

All of our Laboratory's research takes a basic toxicological approach. In our view, however, there is no such a thing as a "toxic agent"--only toxic levels. Our goal is to understand the spectrum of each dose-response curve and its relevance (if any). A major problem, of course, is to determine the proper experimental model for a given study. We choose each model with judicious care before proceeding to the stage of study design.

In attempting to extrapolate from the animal to the human situation, our Laboratory seeks to understand the nature of the dose-response curve and of the dosimetry. In our view, such an understanding is central to a valid risk assessment model. Without knowledge of the nature of the dose-response curve, the statistical significance of an exposure level versus a nonexposure level may fall on the "wrong side" of the extrapolation curve and thus be very misleading. With regard to the comments by Alpen (Chapter 6 of this volume), we feel that an understanding of the mechanism and an understanding of the dose-response spectrum are parallel requirements; both are essential to a valid risk assessment model.

CURRENT EFFORTS

With regard to oxidant air pollutants, our Laboratory's involvement began in 1974 as part of a major Department of Energy project that is primarily concerned with fossil fuel combustion (conventional fuels as well as the new synthetic fuels). Our research focuses on airborne particulates from coal combustion. We employ unique and innovative approaches to collecting samples in the field. Laboratory activities include the generation of aerosols for animal exposure, physical and chemical categorization of samples, and determination of the sample components that carry biological importance in either the pristine (as captured) or degraded (following an interaction with biological membranes) state. The latter activity involves a series of multistage tests. These extend from the more conventional Ames assays (for bacterial mutation) through tests of macrophage function and toxicity and tests of cell transformation (*in vivo* experimentation followed by *in vitro* testing of the transformed or affected cells). A major portion of our program relates to progenitor cells rather than end effect cells; we examine possible interactions with the immunologic system and attempt to relate hormonal and immunologic injury to longer-term carcinogenic potential.

20. PULMONARY EFFECTS OF OZONE IN THE RAT AND MONKEY

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INTRODUCTION

Our work on pulmonary effects of oxidants emphasizes the phenomenon of adaptation (tolerance). Two other primary areas of focus are the evolution of chronic damage resulting from low-level exposure and the effects on lung growth.

CHOICE OF ANIMAL MODELS

Frequently there is much discussion about the appropriateness of a particular animal model for a particular study. There is no such thing as a "perfect model"; in choosing a model, the most important issue is: What questions will be asked of the model? We use two primary species: rats (for statistical purposes and cost effectiveness) and monkeys (for structural/ functional similarities to man and a variety of other anthropomorphic features). We develop our idea with rats and look to monkeys for the more definitive studies.

Regarding lung structural correlates between experimental animals and man, scanning electron micrographs effectively illustrate that the monkey has well developed respiratory bronchioles (like man) whereas the rat does not (Castleman et al. 1975). The importance of this is that the respiratory bronchiole of the monkey is the pulmonary region that is most damaged by low levels of ozone (O_3). At 0.2 ppm, damage is essentially limited to this small airway (Mellick et al. 1977). We are reasonably confident that the bronchiole is also where damage caused by high ambient levels of O_3 occurs in man. We also prefer the monkey because of its usefulness for studies of pulmonary function. We can do repeated experiments; also, the difference in chest wall compliance between the monkey and dog favors the monkey as the species for definitive work.

EXPOSURE REGIMENS

Exposure concentrations are 0.2, 0.5, and 0.8 ppm O_3. These concentrations are measured against the neutral buffered KI calibration. We can factor them to the absolute (UV) standard, but for sake of continuity we use the older calibration. The duration of exposure depends on the purpose of the particular study.

STUDIES OF ADAPTATION DURING CHRONIC LOW-LEVEL EXPOSURE

At 7 d of exposure in normal rats, we find the approximate morphologic no-effect level to be ~0.1 ppm O_3 (Plopper et al. 1979). The effect of prolonged exposure to various O_3 concentrations is dose-related, but also depends on the balance between adaptation and smoldering irritation. Our general hypotheses are commonly held in the field. The first hypothesis is that the centriacinar lesion produced by low-level chronic O_3 exposure can cause and/or exacerbate chronic obstructive pulmonary disease. The outcome depends on the interplay between adaptation and enhancing factors such as infectious and immunologic processes. The concept is straightforward, but the mechanisms involved are complex and largely unknown.

The second hypothesis is that O_3 has a low-level initiation or promotion capability for neoplasia of pulmonary epithelium. On current evidence, we do not believe that O_3 is a significant carcinogen or cocarcinogen. It does produce a larger pool of proliferating cells in small airways in the monkey (and in the mouse), however; thus, a larger cell population may well be at risk. This hypothesis requires further testing.

The terms "adaptation" and "tolerance" are often used in different ways. In the most general sense, adaptation is the process by which tolerance occurs. We prefer to use "adaptation" when referring to changes in pulmonary epithelium. First of all, this usage emphasizes the dynamics of the situation (i.e., what we are trying to understand). Secondly, in O_3 toxicology literature, "tolerance" has been largely preempted by the edemagenic tolerance model, which is not involved in low-level insult. There is another reason for using the term "adaptation." In general pathology, the term refers to cell changes in response to an altered environment, thereby implying that the cells are there to change. "Adaptation" does not apply to situations in which maximal damage occurs initially and cells are either killed or incapable of further response a short time later. Our use of "adaptation" denotes a lessening of detectable damage in spite of continuing insult. In this context, there is a higher capacity for adaptation in the rat than in the bonnet monkey. Here is yet another example of important species differences in biology.

The morphologic manifestation of adaptation is well illustrated by comparing scanning electron micrographs of the rat lung after 7 and 90 d of exposure to 0.2 ppm O_3. Qualitatively, the lung appears to have returned to normal by 90 d, although morphometric studies reveal slightly higher than normal numbers of macrophages. Comparing 7 and 90 d exposure to 0.8 ppm O_3 shows a considerable decrease in the number of macrophages in the lumen, but a reorganization of the centriacinar region (Boorman et al. 1980). This is seen as an intermediate zone resembling a respiratory bronchiole (normally not present in the rat at this age).

Because the damage we see is limited to focal (centriacinar) regions of the lung, an entirely new approach to sampling is needed. Randomizing counts on pieces of minced pulmonary parenchyma is not satisfactory. Methods to sample specifically but without introducing bias must be devised.

With respect to adaptation over a 90-d exposure, the most obvious differences between the rat and the bonnet monkey are the smaller decrease in intraluminal macrophages in affected respiratory bronchioles seen in the monkey and the persistent hyperplasia and hypertrophy of cuboidal, nonciliated bronchiolar epithelial cells also present in the monkey.

Our main current study is a 12-mo exposure of bonnet monkeys to 0.8 ppm O_3, with evaluation of one group of exposed and control monkeys at the end of exposure and of a second group after a 3-mo recovery period. Assessment during the course of exposure is by pulmonary function, including acoustic response (Jackson and Olson 1980), and by pulmonary lavage parameters. These pulmonary function and lavage studies are important both to monitor changes occurring during the 12-mo exposure and to provide a basis of comparison for what might happen in man. Both types of measurement are feasible in humans that have been experimentally or naturally exposed to low concentrations of O_3. The principal terminal assessments will be biochemical and morphometric.

OTHER STUDIES

We have also initiated studies of the effects of O_3 on lung growth and aging in both the rat and monkey. Morphometric evaluations being developed by Dr. Walter Tyler will serve as the most important measures in these studies.

COMPARISON OF EXPERIMENTAL AND EPIDEMIOLOGIC DATA FOR OZONE AND SULFUR OXIDES

One important consideration in inhalation toxicology is that the dose-response curve varies both with time and with the chemical agent. There is some suggestion that the curves for O_3 and sulfur oxides (SO_x) differ considerably. With O_3, experimentally obtained concentrations in polluted urban air cause damage which levels off or lessens with time. Epidemiologically, it is difficult to establish that these levels are in general harmful to exposed human populations. With SO_x, on the other hand, levels that are many times ambient levels are needed to cause an experimental effect, yet the epidemiologic evidence of harm to exposed populations is more satisfying than for O_3. Perhaps the recruitment of a number of different modes of subtle damage occurs gradually until a significant lesion develops. Copollutants in SO_x smogs might also enter into this equation.

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WORKSHOP COMMENTARY

D. L. Coffin: As a pathologist, I was quite impressed with Dr. Dungworth's presentation. Many of these things have been examined by other investigators, but his group is putting them together in a very nice way.

Question: Is it neutral buffered KI or unbuffered KI that you use for O_3 characterization?

D. L. Dungworth: Neutral buffered KI is used to calibrate Dasibi photometric analyzers. The Dasibi analyzers are used for routine monitoring and the data are handled by a computer. We also send the Dasibi analyzers away for occasional checks against absolute UV photometric standards.

Question: How were tissues prepared for scanning electron microscopy? Most of the specimens [shown on slides during the oral presentation] were devoid of any mucous layer in the airways, for example.

D. L. Dungworth: They were fixed by intratracheal perfusion of modified Karnovsky's fixative at 25 cm fluid pressure.

Question: For obtaining the information you presented, how much better is that than, say, optical microscopy of those sections?

D. L. Dungworth: The scanning electron micrograph affords the ability to examine a large block of tissue rapidly and also provides high resolution with large depth of field. This means that one can examine in detail large amounts of airway and alveolar surface.

Question: You made many statements about cell identification and the like. However, you're dealing with a topographic analysis of those cells.

D. L. Dungworth: The scanning electron micrographs were used purely to highlight our studies. We routinely correlate findings by conventional light microscopy, scanning electron microscopy, light microscopy of large 1-μm sections, and transmission electron microscopy of specifically selected regions of pulmonary parenchyma.

21. BIOLOGICAL EFFECTS OF FLY ASH FROM COAL COMBUSTION

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INTRODUCTION

The Laboratory for Energy Related Health Research has maintained a special interest in the airborne particles released from power plants of various types. Our interest extends to particulates from combustion of coal, oil, synthetic fuels, and coal-oil mixtures. This report discusses an EPA-supported study of the fly ash emitted in combination with sulfur dioxide from coal-burning power plants. The study is a collaborative effort with the California Primate Research Center; the large team of investigators is drawn from the fields of biochemistry, pathology, respiratory physiology, and so on. This author's primary interest and involvement are in the dosimetric and exposure phases; the following report emphasizes these aspects but also touches on some of the salient biological results obtained to date.

SAMPLE COLLECTION

In some cases, we directly sample the particles leaving a smoke stack. More frequently, a sample is taken from the hopper of an abatement system (e.g., an electrostatic precipitator). For laboratory exposures, the collected materials are re-aerosolized (see "Exposure Techniques," below).

SAMPLE CHARACTERIZATION

Prior to biological testing, we perform thorough characterization of both the untreated and the re-aerosolized fly ash. Because the particles' aerodynamic behavior is the primary characteristic controlling deposition in the respiratory airways, we give close attention to the parameters that are implicated in aerodynamic behavior. We look very carefully at the size and shape of the particles. Comparisons of untreated versus re-aerosolized fly ash indicate no significant differences in these parameters.

The distribution of electrostatic charge on the particles can also be quite important. In most of our experiments, we reduce the charge to Boltzmann equilibrium before animals are exposed.

Chemical characterization is a major focus. For this, we employ atomic absorption analysis, neutron activation analysis, and particle-induced X-ray analysis. Basically, we find that no two particles have exactly the same chemical composition. Even two particles of the same size will typically show different chemical compositions, particularly with respect to the trace metals present on the particle surface. Certain elements (e.g., zinc, chromium, and arsenic) tend to be concentrated in particles of smaller size due to a primary association with the surface. Comparisons of untreated versus re-aerosolized fly ash indicate no significant differences in chemical composition.

Whether the particles are deliquescent or hygroscopic also has a marked effect on behavior and on particle surface chemical reactions.

BIOLOGICAL TEST SYSTEMS

Our program assesses the biological activity of these materials using short-term bioassay systems (e.g., the Ames assay) as well as inhalation exposures in whole animals. Whole animals include the mouse, rat, monkey, and beagle dog. With regard to whole-animal experimentation, almost all current work with fly ash is performed in rodents; these less expensive species suffice for the necessary range-finding experiments.

EXPOSURE TECHNIQUES

For inhalation studies we generate fly ash using the Wright dust feed mechanism. For several decades this mechanism has been used to expose animals to particles of relatively insoluble materials. Our Laboratory has modified the system by installing a cyclone separator at the exhaust so that larger particles (>2.5 μm in aerodynamic size) are not an appreciable part of the exposure aerosol. Prior to inhalation experiments, the sample material is always size classified to avoid particles that are outside the respirable range for the experimental species. After this initial size separation, the material is loaded as a packed dust cake into the Wright dust feed mechanism. The feeder provides a continuous flow of aerosol as well as a second size separation. Only particles having an aerodynamic equivalent size of <2.5 μm pass into the exposure chamber. A Crypton 85 discharge unit assures that the exposure aerosol is reduced to Boltzmann equilibrium with respect to electrostatic charge.

Fairly large exposure chambers (4 m^3) are available at the California Primate Research Center. Rodents are typically arranged in a monolayer configuration in these chambers. To date, the maximum exposure concentration has been 200 mg/m^3.

A key point about these experiments is that the particles are small enough to be readily inhaled, resulting in fairly large deposition in the lower respiratory tract. At the same time, the conditions are not "dusty" in the sense that the animals are covered with fly ash or the chamber is covered with fly ash. Aerodynamically, the particles are quite stable in this size range.

PRELIMINARY RESULTS

In early screening with the Ames assay (TA-98 strain of Salmonella), the mutagenicity of fly ash collected from the stack of a coal-burning power plant varied with particle size. In general, the larger particles showed a lower number of revertents per milligram of material used in the test. The two smaller size groups (median size of ~2.2 and ~3.5 μm) tended to have higher numbers of revertents in this test.

When we examined the effect of temperature on three strains of Salmonella, we found that heating the samples to >250°C resulted in disappearance of most of the mutagenic activity. Efforts to explain this phenomenon are continuing. Obviously, in the course of combustion fly ash is heated far in excess of 250°C. Probably the as yet unidentified mutagens are formed as the fly ash is released from the stack.

More recently, extensive studies on fly ash collected from the hoppers of electrostatic precipitators (same power plant) showed no mutagenic activity at any particle size. Thus, there is a very definite biological difference between fly ash released from the stack and fly ash collected in the abatement system.

With regard to whole-animal studies, one long-term inhalation exposure (4 mg/m^3, 8 h/d, 180 d) resulted in no remarkable biological changes.

CURRENT STUDIES

Presently we are involved in studies of combinations of sulfur dioxide (50 ppm) with fly ash. In the first experimental series, animals were exposed for ~14 d; there were not many remarkable biological effects. We plan to repeat these experiments at longer time periods.

WORKSHOP COMMENTARY

G. Rausa: A recent article in *Science* states that the mutagenic activity of fly ash comes from methyl sulfate; the association with temperature appears to be about the same. Do you have any technique for ascertaining whether those results are correct?

O. Raabe: One of the coauthors of that paper, Lee Hansen, has been in our Laboratory for about six months on sabbatical leave. I don't believe he has been able to find any methyl sulfate in our aerosols.

G. Rausa: Do you have any conjecture as to what it might be?

O. Raabe: We are working quite diligently to identify the mutagen; it is a very important project to us.

Question: What about polycyclic hydrocarbons? At a conference a couple of summers ago, Paul Morrow pointed out that benzopyrene, which is the surrogate for the rest of the polycyclic hydrocarbons, comes out of the stack in a volatile state and then condenses on the particles as the plume cools. Not finding it in the hopper and finding it on the true fly ash would seem to fit with that.

O. Raabe: To be mutagenic, benzopyrene must be activated. In our results, no activation effect is apparent. So it may be something "from" benzopyrene, but it isn't "just" benzopyrene.

The problem with our mutagenic fly ash is that it is very rich in organic materials, and polycyclic aromatic hydrocarbons are not a major part of that organic constituency. Dr. Kimball of our Laboratory has been working with this material; she finds, I believe, that there is so much organic material (in comparison to polycyclic hydrocarbons) that it becomes very difficult to identify polycyclic hydrocarbons as the culprit agent.

Question: Was the ash studied by the Utah group collected way down where it was cool, or was it more reflective of the stack?

O. Raabe: I think they collected it somewhere downstream, outside the power plant. Ours was collected from the base of the smoke stack. Thus, there is quite a difference.

Question: How close is the geometric surface to the gas absorption surface on these spheres?

O. Raabe: That's a good question; unfortunately, I don't have the data with me. Our measurements show that the gas absorption surface is greater than the geometric surface, but it's not remarkably greater. For the very large particles, there are quite a few hollow spheres and spheres that are tightly packed with little particles. These features are not seen in particles of the small respirable range, however. The small particles have a rough surface but no obvious pores.

22. OVERVIEW OF RESEARCH AT THE INHALATION TOXICOLOGY RESEARCH INSTITUTE

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INTRODUCTION

This report presents a brief overview of the Inhalation Toxicology Research Institute. Included are descriptions of the Institute's resources and of the current research program. Chapters 23 through 26 of this volume present more detailed information on four projects relating to oxidant air pollutants.

OPERATION AND FUNDING

The Inhalation Toxicology Research Institute is operated by the Lovelace Biomedical and Environmental Research Institute for the Department of Energy under the Assistant Secretary of the Environment. The parent foundation is the Lovelace Medical Foundation. Most (currently, 80%) of the Institute's funding comes from the Department of Energy, but several projects are funded by other governmental agencies. Funding from these other agencies (EPA, the

Nuclear Regulatory Commission, and the Department of Defense) is made possible through interagency agreements with the Department of Energy.

FACILITIES AND STAFF

The Institute is located on a 138-acre site on Kirtland Air Force Base south of Albuquerque, New Mexico. Facilities include ~215,000 ft^2 of floor space; the current replacement value is somewhere on the order of $20 million. All support services are provided onsite; the Institute is essentially self-contained.

There is maintenance housing for ~15,000 small animals; the exact number housed, of course, depends on the mix between mice and larger species. At present, there are facilities for housing ~2,000 dogs. The Institute maintains a breeding colony of beagle dogs; mice, rats, and Chinese hamsters are also bred. The current production capability is ~4,000 mice and ~2,000 rats per month, and ~400 dogs per year. Actual production depends on research needs at any given time.

In general, the Institute at full strength employs ~250 people. The present total staff of 227 includes ~50 doctoral-level personnel and a mix of disciplines: ~50% in the life sciences (biology or medical-related degrees), ~25% in biophysics and biomedical engineering, and ~25% in the physical and engineering sciences (including mathematical modeling and risk assessment).

RESEARCH CAPABILITIES

For several years the Institute has maintained strong capabilities in the area of inhalation exposure to both radioactive and nonradioactive materials and aerosols. There is also considerable analytical capability, not only in chemistry but also in radioanalysis.

Another Institute strength is the diversity of biological end points that can be evaluated. Capabilities encompass not only the clinical end points (e.g., physical examinations, radiography, clinical chemistry, and hematology) but also organ function (e.g., respiratory function, mucociliary clearance, and lung defense mechanisms). In other words, it is possible to evaluate organ function as well as whole body function.

Deposition and retension of inhaled materials (including aerosols, vapors, and gases) continue to be important end points in determining critical doses to tissues. In the area of immunology, there is a strong program focusing on the impairment in immune mechanisms caused by inhaled environmental pollutants (particularly in the lung-associated lymph nodes) and on determination of the immune pathways involved.

Mutagenesis and carcinogenesis testing capabilities, of course, are related, and one of the Institute's major strengths is the ability to go from *in vitro* mutagenicity studies to whole-animal carcinogenicity studies. Current protocols involve bacterial and mammalian cell mutagenicity testing followed by long-term studies of cancer development in animals. In the field of cytogenetics, there is a group looking directly at the genetic transformations that take place in mammalian cells and scoring chromosome damage.

Mortality, morbidity, and pathology are common end points in any long-term animal study. The Institute employs not only gross morphology and light microscopy, but also transmission and scanning electron microscopy. Quantitative morphometric measurements are also commonly used.

The Institute's capabilities in evaluating biochemical end points are particularly strong. One focal area involves lung lipid chemistry, Type II cell metabolism and injury, and lung surfactant studies. Another strong area is the biochemistry of lung connective tissue.

CURRENT PROJECTS

Approximately 50% of the Institute's current research program relates to nuclear power production. Studies involve both the fission products and the transuranics such as plutonium and other alpha emitters. Both short- and long-term studies are ongoing in these areas.

The Fossil Fuel program evaluates effluents from stationary sources: coal combustion and coal gasification. In the area of coal combustion, both conventional combustors and fluidized bed combustors are undergoing evaluation.

In the Mobile Sources program, the main effort currently relates to the study of diesel exhaust. A five-part program begins with characterization of the exhaust material and extends through animal life-span studies.

The Solar program is the only area involving studies that are not primarily inhalation-oriented. Because of the possibility for heat transfer fluids to enter the potable water supply, Institute researchers are studying the toxicology of these materials. Studies focus on the ingestion and contact toxicology of heat transfer fluids.

The Conservation program's main effort relates to the study of fibers (both natural and man-made) used in insulating processes.

The spectrum of materials that can be analyzed ranges from solid particles to droplets, vapors, and gases. Materials currently analyzed include conventional combustor and fluidized bed combustor fly ash, diesel particulates, ammonium sulfate (as either a solid particle or droplet), sulfuric acid, and irritant or oxidant vapors and gases. Most of the current studies are oriented towards single agents. As the need is recognized, the Institute will undertake studies of combinations of these materials, bearing on the problem of what happens when ambient mixtures are inhaled.

Studies can also progress in the opposite direction. For example, efforts in the diesel exhaust study started with diluted raw exhaust but now involve some of the individual components.

In subsequent chapters of this volume, other authors from the Inhalation Toxicology Research Institute report results of studies that have been concluded, review ongoing efforts, and emphasize proposed directions for future research. Henderson (Chapter 23) discusses a project in which the primary goal was to develop and demonstrate the usefulness of cellular damage indicators for lung injury. The techniques developed in this recent, exciting work show much promise as early detectors for lung cell injury and death.

Pickrell (Chapter 24) outlines a program on inhaled nitrogen dioxide that includes both short- and long-term studies. Silbaugh (Chapter 25) discusses a program to examine the structural and functional changes associated with inhaled sulfates, again including both short- and long-term studies.

Finally, Wolff (Chapter 26) reviews studies primarily related to the clearance of inhaled particles in the lung and the effect of inhaled sulfates on the pattern (velocity or rapidity) of clearance of inhaled materials. This is actually a study of lung defense mechanisms.

23. CELLULAR (IN VIVO) AND BIOCHEMICAL CHANGES FOLLOWING INHALATION OF ACID SULFATES: RAPID SCREENING TESTS TO DETERMINE THE PULMONARY RESPONSE TO COMBINED POLLUTANT EXPOSURES

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PROBLEM

A major lack in the information required to regulate air pollutants is knowledge of how various pollutants interact to produce synergistic, additive, or negating effects in the exposed populace. To expose experimental animals to single component aerosols on a long-term basis is expensive; adding double or triple components increases the cost even more. One approach to the problem is to use rapid *in vitro* screening tests, which cost less and yield results quickly. However, for studying pollutant interactions, an *in vitro* approach does not take into account the interactions that affect site of deposition, clearance, retention, and (consequently) pollutant dose to tissue. Epidemiology assesses real-world pollutant mixtures but cannot isolate the effects of individual components of those mixtures. What is needed is a rapid bioassay that forms a bridge between the *in vitro* studies and the long-term *in vivo* and epidemiologic studies.

APPROACH

Our laboratory uses bioassays that involve short-term *in vivo* inhalation exposures followed by rapid evaluation of pulmonary response by a variety of indicators of early lung damage. Our past use of these bioassays to measure pulmonary response to single pollutants is summarized below.

Lavage Fluid Analysis

Previous work showed that analysis of saline lung washings of exposed animals can be used to detect an inflammatory response in the lung (Henderson et al. 1978a, 1978b, 1979a, 1979b). Extracellular lactate dehydrogenase in the airway fluid indicates increased cell membrane permeability; lysosomal enzymes indicate phagocytic activity; increased sialic acid indicates an irritant response in the upper airway; increased soluble protein in the airways indicates damage of the capillary-alveolar barrier; and an influx of neutrophils is consistent with an inflammatory response. Biochemical and cytological changes in lavage fluid correlated well with morphological indicators of an acute inflammatory response in Syrian hamster lungs exposed to a nonionic surfactant (Henderson et al. 1978a) and to metal salts (Henderson et al. 1979a, 1979b). The most sensitive indicator of the multifocal lung damage (terminal bronchiolitis) characteristic of oxidants was an increase in the polymorphonuclear cells in lavage fluid from hamsters exposed to nitrogen dioxide (NO_2) (DeNicola et al. 1979). Rats exposed to sulfuric acid (H_2SO_4) mist showed an increase in the sialic acid content of the lavage fluid as well as speeding of mucociliary clearance (Henderson et al. 1978c).

^{14}C-Thymidine Incorporation in Lung Cells

Pulse labeling of Chinese hamsters with ^{3}H-thymidine demonstrated an increased uptake of radiolabel in the lungs of animals exposed to NO_2 (Hackett 1979). Tissue oxidizer analysis of ^{3}H retention by the lung could be used as a rapid screen for cellular damage. In animals showing the greatest response, autoradiographic techniques could be applied to portions of the tissue to determine the actual site of damage.

Lipid Synthesis

One indicator of cellular damage is an increased synthesis of membrane lipids during repair processes. An increased uptake of ^{14}C-palmitate into membrane lipids was detected in lung cells from animals exposed to 5 ppm NO_2 for 8 h (Pfleger and Rebar 1978). The same label would also detect increased synthesis of the surfactant lipid, dipalmitoyl lecithin. This lipid is synthesized by the Type II epithelial cell, which is known to proliferate in response to the most common type of lung injury--loss of the Type I epithelial cell.

PLANNED RESEARCH

The bioassays described above have been used to detect pulmonary response to single pollutants. We propose to extend their use to detect the effects of combinations of pollutants.

We plan to use the Charles River Hartley guinea pig because of our previous experience with this animal's response to H_2SO_4 (Silbaugh et al. 1978). Animals will be exposed to four combinations of NO_2 and H_2SO_4 (5 ppm NO_2 plus 1.0 mg/m^3 H_2SO_4; 1 ppm NO_2 plus 1.0 mg/m^3 H_2SO_4; 5 ppm NO_2 plus 10 mg/m^3 H_2SO_4; 1 ppm NO_2 plus 10 mg/m^3 H_2SO_4) and to the same levels of the individual components. Control animals will be exposed to room air. Exposures will be for 6 h on 2 subsequent days. The animals will be evaluated at 48 h after initiation of the exposure, which has been identified as the time of peak response to NO_2 (DeNicola et al. 1979; Hackett 1979; Pfleger and Rebar 1978). The end points to be measured include thymidine and palmitate uptake into lung tissue as well as lavage fluid parameters. The lavage fuid parameters will include lactate dehydrogenase, acid phosphatase, sialic acid, soluble protein, glutathione peroxidase and reductase, fibrin degradation products, and total and differential cell counts. In addition to these end points, we will perform light microscopic evaluation of slices of deep lung tissue, trachea, larynx, and nasal turbinates. In animals shown by the screen to be most affected by exposure, autoradiographs of the ^{3}H-thymidine labeled tissue and scanning electron micrographs of the tissues will be obtained.

Upon completion of this study, we will examine the effects of combined exposure to H_2SO_4 (1 mg/m^3) and fly ash (5 mg/m^3) from fluidized bed combustion of coal. The final experiment will examine the effects of combined exposure to all three pollutants: NO_2 (5 ppm), H_2SO_4 (1 mg/m^3), and fly ash (5 mg/m^3).

This research should aid in validating the screening system; the higher pollutant levels have been included to meet this validation need. The major result of the experiment will be to determine if any synergistic effects occur in combined acute exposures to low levels of the described pollutants. This determination should contribute to the groundwork for any regulatory decisions based on combined effects from pollutant mixtures.

ACKNOWLEDGMENTS

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WORKSHOP COMMENTARY

J. D. Hackney: In the ^{3}H-thymidine labeling, what was labeled, and how? Was this by autoradiography or was this extracted from the lung?

R. F. Henderson: This was by an autoradiographic technique which showed labeling of epithelial cells. But autoradiography is not a screening tool. It is a very slow procedure. For a screening tool we propose to use the first step of our autoradiography studies in which a tissue oxidizer is used to determine which animals have actually taken up label. The tissue oxidizer oxidizes the ^3H and ^{14}C in the tissues to ^3H_2O and $^{14}CO_2$, which can be collected and counted in a liquid scintillation spectrometer.

D. E. Gardner: In your studies showing increases in polymorphonuclear cells, did you look at the functioning of these polymorphonuclear cells, their phagocytic capabilities, or the macrophages--

R. F. Henderson: No. The macrophages appeared activated (that is, they were enlarged and had large inclusion bodies), but we made no measurements other than to observe the morphological changes.

D. E. Gardner: Did you find a decrease in macrophages with the increase in polymorphonuclear cells following NO_2 exposure?

R. F. Henderson: Both the macrophages and the polymorphonuclear cells in the lavage fluid increased.

24. RESPIRATORY TOXICOLOGY OF NITROGEN OXIDES

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PROBLEM

Assessing the health effects of nitrogen oxides (NO_x) provides information that is necessary in the regulatory process. Although short-term inhalation of high concentrations of nitrogen dioxide (NO_2) is known to damage both pulmonary alveolar macrophages and Type I epithelial cells, the health effects of inhaling environmental levels of NO_2 for long periods of time have not been adequately defined. Little is known concerning the health effects of inhaling NO_2 in combination with other pollutants such as ozone (O_3) or fly ash. Also, the effects of inhaling other oxides of nitrogen (nitric oxide, nitrates, nitrites, peroxyacyl nitrate, nitrous or nitric acid, nitrosamines) have not been studied extensively.

Lung tissue exposed to a variety of NO_2 levels seems to adapt to this insult. The mechanism and extent of the adaptation and its relation to the animal's health status are poorly understood. Indicators for a variety of changes are required to assess the extent of pulmonary injury, adaptation, and residual injury. Biochemical indicators of damage, although quite sensitive, must be related to cellular and morphological changes to understand lung injury following NO_2 inhalation. Of special importance are those parameters which persist long after pulmonary injury has ceased. These are the biomedical determinants of the resulting pulmonary injury.

The experiments described below were directed toward defining the NO_2 dose causing acute effects and the consequence of those effects for development of chronic pulmonary injury. Nitrogen dioxide damage to pulmonary alveolar Type I cells is reflected in cell necrosis, pulmonary edema, an influx of polymorphonuclear (PMN) leukocytes, and an increased pulmonary distensibility. This damage leads to turnover of extracellular matrix, heightened immune response in the regional lymph nodes from increased access of antigen to interstitial tissue, and a proliferation of Type II alveolar pneumocytes. Most indicators of damage return to normal and the lung seems to "adapt" to the NO_2 injury. Spindle cell proliferation, continued low-level inflammation, continued increased pulmonary distensibility, and increased mean linear intercepts and pulmonary collagen suggest that incomplete pulmonary adaptation to NO_2 exposure may lead to chronic pulmonary injury.

Future work will define the relation of incomplete adaptation to development of chronic pulmonary injury from NO_2 levels relevant to smog-filled urban atmospheres. The relation of NO_2 and O_3 to pulmonary injury will be explored (again at levels relevant to environmental exposures).

Finally, acute studies will assess the toxicities of other oxides of nitrogen in comparison to NO_2.

APPROACH

The effect of inhaling NO_2 at several levels was studied in Chinese and Syrian hamsters and Fischer 344 rats. Chinese hamsters were used in cytokinetic studies so that the resulting data could be correlated with data from parallel analyses of lung chromosomes in the same species. Chinese hamsters were selected for the lung chromosome analysis because of the species' simple chromosome pattern. Syrian hamsters had been determined to be free of long-term lung disease in earlier parallel studies, and were therefore selected. Later, Fischer 344 rats were used because of a longer lifespan in comparison to the Syrian hamsters. Where comparisons between species were made, NO_2 seemed to induce similar series of morphological, cytological, and biochemical events. The significance of each change is discussed below.

Exposure of Fischer 344 rats to 20 ppm NO_2 for 48 h led to damaged alveolar Type I cells (Pickrell et al. 1978). These changes were reflected by increased airway lactate dehydrogenase (LDH), suggesting pulmonary cell damage, and by increased airway alkaline phosphatase, suggesting pulmonary cell necrosis. Increased airway protein and trypsin inhibitory capacity suggested pulmonary edema. Pulmonary edema in the airways suggested both increased vascular permeability and an interruption of the tight junction of alveolar Type I cells due to cell damage. This Type I cell damage led to an influx of PMN leukocytes into the airway and an increased turnover of collagen of the extracellular matrix by 14 d. This change reflected collagenolysis and proteolysis of pulmonary interstitium probably from exposure to airway enzymes from inflammatory cells (macrophages and PMN leukocytes). Such exposure was possible, since damage to alveolar Type I cells had occurred. Increased parenchymal alkaline phosphatase and neutral protease were consistent with the terminal bronchiolitis observed at that time. Results from other studies in rats and Syrian hamsters suggested damage to alveolar Type I cells.

In a second study, exposure of adult male Fischer 344 rats to 26 ppm NO_2 for 24 h increased the number of anti sheep red blood cell plaques formed by lung-associated lymph nodes (LALN). This change may have reflected an altered load to these nodes due to pulmonary Type I cell damage. In another study, Syrian hamsters exposed to 12 ppm NO_2 for 48 h had a mild necrotizing terminal bronchiolitis with increased airway PMN leukocytes. In another experiment, Syrian hamsters exposed to 5 ppm NO_2 for 8 h showed increased quantities of viable airway granulocytes by 48 h after initiation of exposure, suggesting damage to alveolar Type I cells and recruitment of inflammatory cells.

Necrosis of pulmonary alveolar Type I cells led to hyperplasia of pulmonary alveolar Type II cells (Evans et al. 1977). In Chinese hamsters exposed to 15 ppm NO_2 for 24 h, an increased labeling index for ^{3}H-thymidine was observed by 24 h and persisted to 3 weeks after exposure (Hackett 1979). Type II cells were observed twice as frequently in the terminal bronchioles as in other pulmonary areas. The Type II cell cycle time was reduced from 26 d to 3 d. These changes were consistent with terminal bronchiolitis, Type I cell death, and Type II cell proliferation. Epithelial cells lining small airways and alveoli were more susceptible to NO_2 exposure than were bronchial and tracheal epithelia (Hackett 1979). Syrian hamsters exposed to 12 ppm NO_2 for 48 h showed bronchiolar epithelial hyperplasia. Fischer 344 rats exposed to 20 ppm NO_2 for 48 h had terminal bronchiolitis and changes suggestive of Type II cell hyperplasia (Pickrell et al. 1978). Both studies were consistent with damage to alveolar Type I cells in terminal bronchiolar areas leading to Type II cell hyperplasia.

Type II alveolar cell hyperplasia was associated with alterations in synthesized lipids probably destined for cell membranes. Cells from an "enriched Type II cell fraction" of lungs of Syrian hamsters exposed to 15 (Pfleger et al. 1980), 5, or 1 ppm for 8 h had increased incorporation of radiolabel into unsaturated phosphatidyl choline, reflecting increased production of membrane lipid. These changes were consistent with increased production of cell membrane lipid by Type II cells (exposed to 1 ppm NO_2 for 8 h), a step which must precede hyperplasia of Type II alveolar pneumocytes.

When rats were exposed to 20 ppm NO_2, most indicators of damage returned to normal by 2 mo after initiation of exposure (Pickrell et al. 1978). The lung seemed to "adapt" to continued exposure. Return of airway granulocytes, tissue alkaline phosphatase and acid protease, and tissue LDH to control levels by 2 mo after initiation of exposure suggested that parenchymal tissue inflammation was no longer present. Return of airway LDH and alkaline phosphatase to control levels suggested that parenchymal cell damage and necrosis were no longer occurring. Finally, return of airway collagen to control levels suggested that disruption of the extracellular matrix had ceased. This was consistent with a reduction in Type I alveolar cell necrosis and an "adaptation" of the lung to continued NO_2 exposure. Lungs of Syrian hamsters exposed to 12-22 ppm NO_2 had returned to normal by 21 d after initiation of a 48-h exposure. By 7 d after exposure of Fischer 344 rats to 26 ppm NO_2 for 24 h, the ability of LALN to form plaques following challenge with sheep red blood cells had returned to the control level, suggesting that the antigen load to LALN had returned to normal. This was consistent with cessation of damage to Type I alveolar cells and with lung adaptation to NO_2.

Several parameters remained altered (relative to control animals) in Fischer 344 rats exposed to 20 ppm NO_2 for 2 mo. These changes suggested that any "recovery" to that exposure was incomplete by 10 mo after stopping exposure. Chronic pulmonary injury had presumably occurred. Pulmonary distensibility at 20 cmH_2O was increased relative to controls at 6.5 mo but not at 12 mo after initiation of NO_2 exposure. This morphometric parameter slowly adapted, suggesting that pulmonary inflammation or alveolar distension lessened. Persistence of increased neutral protease throughout the study suggested that chronic low-level inflammation continued 10 mo after NO_2 exposure ceased. The increase of mean linear intercept observed 2-12 mo after initiation of exposure was not accompanied by histological evidence of septal destruction or emphysema. The thinning of walls and increased alveolar size (histologically within normal limits) observed 12 mo after initial NO_2

exposure were consistent with increased linear intercept and may have represented a reduction in elastic recoil. The degree to which elastin quantity or metabolism were altered was not determined. By 14 d after initiation of NO_2 exposure, alveolar walls were subtly thickened by apparent spindle cell proliferation suggestive of early fibrosis, although collagen content was normal. Later, collagen content was increased from 2-12 mo after initiation of NO_2 exposure, although no histological evidence of fibrosis was present. One may speculate that these changes were consistent with collagen replacement of alveolar elastic tissue, loss of elastic recoil, and increased mean linear intercepts. If the replacement was sufficiently diffuse, no histological evidence of wall destruction might have been noted. Certainly these changes suggest that incomplete "adaptation" to pulmonary injury from NO_2 exposure may lead to chronic pulmonary injury not resolved 10 mo after cessation of exposure. Such chronic pulmonary injury might occur even in the absence of septal destruction. The relation of pulmonary "adaptation" and chronic pulmonary injury to lower levels of NO_2 with intermittent "spike" levels relevant to concentrations encountered in smog should be of considerable interest.

PLANNED RESEARCH

Future work assessing the toxicity of inhaled NO_x will bear on important scientific questions relevant to the goals of EPA. Further work with NO_2 will address the still unanswered questions of (1) a minimal effects or low-risk level, (2) adaptation to intermittent NO_2 spikes simulating urban levels, and

(3) the effects of NO_2 in older animals or in animals with preexisting disease. The most important and relevant question is the mechanism, extent, and consequences of adaptation to intermittent spikes of NO_2 superimposed upon a 3 to 5 fold lower maintenance level of NO_2.

A second important area for which there is little information is the interaction of NO_2 with such other pollutants as O_3, peroxyacyl nitrate, fly ash, nitric oxide, and oxides of sulfur. The most frequent interaction of NO_2 is with O_3; these pollutants frequently coexist in smog. Both have considerable potential for producing chronic pulmonary injury. The pulmonary system seems to at least partially "adapt" to either pollutant.

Another important scientific need is an assessment of the relative toxicities of other oxides of nitrogen, peroxyacyl nitrate, nitrosamines, nitrates and nitrites, and nitric and nitrous acid. Peroxyacyl nitrate is a potent oxidant. Preliminary reports suggest that it has the same approximate relative toxicity as O_3 and greater relative toxicity than NO_2, although ambient atmospheric conditions seem quite low. Nitrosamines are known carcinogens, but ambient atmospheric conditions appear considerably lower than even those of peroxyacyl nitrate.

The mechanism, extent, and consequences of pulmonary adaptation to NO_x will be studied using a maintenance level of 1 ppm NO_2 for 24 h/d, 5 d/week. A spike of 5 ppm NO_2 will be superimposed on the 1 ppm level in a second group of animals. This spike will occur 2 times/d, to simulate urban pollution conditions. These groups will be compared to a third group of animals exposed to 5 ppm NO_2 5 d/week and to a control group housed in exposure chambers. Sacrifices will occur from 2 weeks to 2 yr after the initiation of exposures. The study will employ ~250 Fischer 344 rats.

End points will relate to the general areas of pathology, morphometry, physiology, and biochemistry. The pathological assessment will include histopathology and electron microscopy (both scanning and transmission electron microscopy). Morphometric end points will include pulmonary distensibility (displaced volume of lung fixed at 24 cmH_2O/kg body weight) and mean linear intercept. Internal surface areas will be calculated. Pulmonary physiology will include measurement of static compliance in excised lungs and measurement of other respiratory functions in selected animals. Biochemical measurements will include lipoperoxidation, glutathione peroxidase and reductase, acid and alkaline phosphatase, LDH and glucose-6-phosphate dehydrogenase, protein, trypsin inhibitory capacity, protease activity as a function of pH, and collagen and elastin metabolism.

The effect of inhaling NO_2 and an associated pollutant, O_3, is an important scientific question. Fischer 344 rats will be used to establish a dose response and interaction. The end points to be studied are described above. Six groups will be exposed to NO_2, O_3, or both: (1) 1 ppm NO_2, (2) 5 ppm NO_2, (3) 0.25 ppm O_3, (4) 1 ppm O_3, (5) 1 ppm NO_2 plus 0.25 ppm O_3, and (6) 5 ppm NO_2 plus 1 ppm O_3. An equal sized group of controls will be housed in chambers and exposed to ambient filtered air. Each group will contain ~140 rats. Serial sacrifices will occur from 1 mo to 3 yr after initiating exposure. Approximately 60 rats will be retained for lifespan studies.

Assessement of the relative toxicities of other oxides of nitrogen will be accomplished in short-term acute experiments. These dose-response studies will employ at least two levels, one quite high. Responses will be observed in days to weeks as opposed to years (except for known carcinogens, for which observations may continue for up to 1 yr). The relative toxicity information will be combined with estimates of environmental concentrations in an effort to predict relative hazard. The compound with the greatest relative hazard will be selected for a long-term toxicity study. We consider peroxyacyl nitrate and nitrosamines to be the most relevant pollutants for initial assays. Peroxyacyl nitrate is reported to be a potent oxidant: preliminary work suggests its toxicity to exceed that of NO_2 and to approach that of O_3. To date, peroxyacyl nitrate has received little attention because its ambient concentrations appear to be extremely low. Nitrosamines are known to be potent carcinogens. However, their ambient concentrations appear to be orders of magnitude below even those of peroxyacyl nitrate. Methods of detecting and quantifying peroxyacyl nitrate and nitrosoamines continue to be improved, and care must be taken in interpreting measured atmospheric levels.

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WORKSHOP COMMENTARY

P. E. Morrow: You mentioned something about the antigenic burden of the lymph nodes. Would you expound on that?

J. A. Pickrell: Dr. David Bice of our laboratory performed a study on pulmonary immunology and NO_2. Rats were exposed to 26 ppm NO_2 for 24 h. Use of sheep red blood cells to stimulate the lung immune response in regional lymph nodes led to increased plaque formation in LALN. The increase in plaque formation peaked at 2 d, was reduced at 3 d, and had returned to normal by 7 d after exposure. His interpretation was that the increased load to the LALN was due to interruption of the Type I alveolar pneumocyte barrier, that the reduction represented Type II cell proliferation and phagocytosis of peroxyacyl nitrate, and that normal pulmonary architecture had been restored by 1 week after exposure.

P. E. Morrow: What does that have to do with the Type I cell barrier?

J. A. Pickrell: The sheep red blood cells were introduced into the lung and the lymph nodes were stimulated. Bice interpreted this to mean that the epithelial integrity of the lung was interrupted; I think he had some histopathology to back this up. He concluded that more sheep red blood cells were therefore available to the lymph nodes and that the lymph nodes were simply responding to an increased load in a normal immunologic manner.

P. E. Morrow: I follow the antigenic stimulation concept but I don't think the Type I integrity follows.

There is another point that I wish to clarify. In all of the studies in which you've done thymidine incorporation and have looked for proliferative changes and so on, you did not mention the Clara cell in these rodents. I thought that this was a very prominent feature of small airway effects: that there would be damage in that area and there would be Clara cell proliferation. But it hasn't been mentioned; instead, the discussion focused on Type II cells and basal cells. Did you look specifically at the Clara cell?

J. A. Pickrell: It was looked at by Dr. Nora Hackett of our laboratory; the predominant response she saw was a Type II alveolar pneumocyte proliferation.

P. E. Morrow: Isn't the Clara cell alleged to be the progenitor cell in the small airway epithelium?

J. A. Pickrell: Yes.

R. F. Henderson: I don't believe Dr. Hackett distinguished the Clara cell, but merely indicated nonciliated airway epithelium.

Question: Did Dr. Hackett differentiate what you're calling "Type II" from the Clara cell?

J. A. Pickrell: She differentiated Type II cells from nonciliated and ciliated airway lining cells.

Question: What is your understanding of the ambient concentrations of O_3 and NO_2? You mentioned 1 and 5 ppm as "approaching ambient concentrations."

J. A. Pickrell: In the Los Angeles Basin, O_3 might approach 0.3 ppm to 0.5 ppm; NO_2 seldom exceeds 1 ppm and is usually ~0.5 ppm. The Los Angeles Basin, I believe, displays one of the higher concentrations in the country. Does that answer your question?

Comment: Yes. My comment would be that 5 ppm is not anywhere near ambient concentration.

J. A. Pickrell: That's correct. 1 ppm would be.

Comment: I don't believe that there are any monitoring data anywhere close to 1.5 for NO_2--

J. A. Pickrell: I believe in some of the NO_2 reports I've seen it has approached 1, but you're right: it's more nearly 0.5.

D. E. Gardner: I'm sure you're familiar with Dr. Gus Freeman's work with NO_2 in which rats and monkeys were exposed for a lifetime. Freeman very methodically described many of the effects that you have reported, not only with NO_2 alone but also with NO_2 plus O_3 (related to what you're proposing). Is the purpose of your studies to confirm his? What have you added to Freeman's studies?

J. A. Pickrell: I don't think he used spikes of NO_2 to simulate urban conditions.

D. E. Gardner: I'm referring to your NO_2-O_3 study.

J. A. Pickrell: Freeman's NO_2-O_3 study, if my recollection is correct, used somewhat higher levels than ours.

D. E. Gardner: The lowest level was 2 ppm.

J. A. Pickrell: Freeman's NO_2 level was 2 ppm, but the NO_2-O_3 study was done at a somewhat higher level, if I remember correctly.

Comment: The O_3 level was 0.6 ppm.

S. V. Dawson: In view of your use of high and low levels, what do you consider to be high and low levels for the peroxyacyl nitrate and nitric acid exposures that you're planning? Also, which type of peroxyacyl nitrate will be used, and how will you obtain it?

J. A. Pickrell: I haven't decided what type of peroxyacyl nitrate will be used.

When they're detected at all, peroxyacyl nitrate levels are in the ppb range. The effects levels that have been reported so far are in the ppm range. Therefore, we propose to start where effects levels have been reported and work down toward ambient levels. As our high level, we will use a concentration just below the concentration at which effects have been reported. For our low level, we will use a level an order of magnitude lower.

D. E. Gardner: Have you begun to generate and monitor peroxyacyl nitrate?

J. A. Pickrell: No.

D. E. Gardner: Our recent experience suggests that you may encounter real problems. Peroxyacyl nitrate is not very toxic. In most model systems it presents real problems. You might talk with the Illinois Institute of Technology Research Institute [Life Science Division, 10 West 35th St., Chicago, IL 60616]; they did the work for us, and talking with them might give you a head start in this area.

J. A. Pickrell: Thank you.

25. ACUTE-CHRONIC LOSS OF LUNG FUNCTION FOLLOWING INHALATION OF ACID SULFATES

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PROBLEM

The existence, nature, and importance of health effects resulting from the inhalation of acid sulfate aerosols (alone and in combination with other fossil fuel combustion byproducts) are being addressed. This project provides information relevant to the setting of sulfate and fine particulate standards.

APPROACH

Toxicity of Sulfuric Acid Aerosols in the Guinea Pig

We conducted dose-response studies of the mortality of Hartley guinea pigs exposed for 8 h to sulfuric acid (H_2SO_4) particles of either 0.4 or 0.8 µm in diameter. A total of 96 animals were divided into groups and exposed to 43, 83, or 109 mg/m^3 of the 0.4-µm aerosol or 21, 32, or 43 mg/m^3 of the

0.8-µm aerosol. Marked differences in lethality of the two aerosols were observed. The LC_{50} for the 0.8-µm aerosol was ~30 mg/m^3, while an LC_{50} for the 0.4-µm aerosol was not reached even at 109 mg/m^3.

Effects of Sulfuric Acid Aerosols on Pulmonary Function in the Guinea Pig

Using Hartley guinea pigs, we completed studies of the acute respiratory function effects of inhaled 1.0-µm H_2SO_4 particles. A total of 57 animals were exposed for 1 h to concentrations of 0, 1.2, 1.3, 14.6, 24.3, or 48.3 mg/m^3; respiratory patterns and breathing mechanics were measured during exposure. The observed effects differed from the graded dose-response relationship previously reported by Amdur et al. (1978). Functional responses reflecting airway constriction were either absent (nonresponsive animals) or overwhelming (responsive animals). The proportion of responsive to nonresponsive animals increased with exposure concentration, but the magnitude of pulmonary function change was similar for responsive animals regardless of concentration. Our results suggest that the Hartley guinea pig reacts to inhaled H_2SO_4 with an essentially all-or-none airway constrictive response, and that there is considerable variation in the concentrations at which different animals develop the response.

Animal Strain Differences and Adaptation to Acute Sulfuric Acid Exposure

Twelve guinea pigs of each of the following strains were exposed to 30-36 mg/m^3 of 1.0-µm H_2SO_4 aerosol: Charles River Hartley, Camm Hartley, Camm

English Shorthair, and NIH Strain 13. Half of the exposures involved a gradual increase to the above concentration over a 1-h period, followed by a 1-h exposure at a steady concentration. The remaining exposures were conducted for 1 h with an immediate change from clean air to the desired concentration. The degree of labored breathing during exposure was scored visually. The NIH Strain 13 guinea pigs developed more severely labored breathing than the other strains, but these animals were later discovered to have respiratory infections. All strains had less labored breathing when H_2SO_4 concentrations were increased gradually rather than suddenly, suggesting partial adaptation.

Sulfuric Acid and Nitrogen Dioxide Induced Alterations in the Guinea Pig's Sensitivity to Histamine Aerosol

Hartley guinea pigs exposed for 1 h to an H_2SO_4 aerosol of 1 µm in mass median aerometric diameter (MMAD) or to nitrogen dioxide (NO_2) gas were examined for alterations in airway responsiveness to inhaled histamine. Concentrations of H_2SO_4 ranged from 4 to 40 mg/m^3; concentrations of NO_2 ranged from 7 to 146 ppm. One group of animals exposed to filtered room air served as the control. Animals were exposed to stepwise increased concentrations of ~0.6-µm histamine aerosol 2 h prior to pollutant exposure and at 0, 2, and 19 h after exposure. The histamine concentration required to produce a 50% decrease from pre-challenge dynamic lung compliance was used as a measure of airway sensitivity. All animals exposed to NO_2 exhibited an increase in histamine sensitivity immediately after exposure; the magnitude of this increase was concentration dependent. We observed a dramatic return of the sensitivities of most animals toward base-line values at 2 h after exposure; however, the sensitivities of several animals remained elevated at 19 h after exposure. Animals exposed to H_2SO_4 exhibited major increases in histamine sensitivity only if labored breathing developed during exposure. We concluded that both NO_2 and H_2SO_4 alter airway sensitivity in the guinea pig, but by apparently different mechanisms.

PLANNED RESEARCH

Alterations in the Guinea Pig's Sensitivity to Histamine Following Acute Nitrogen Dioxide Exposure at 5 ppm

The above results demonstrated that major alterations in the guinea pig's airway responsiveness to inhaled histamine could be produced by 1-h exposures to 40 ppm NO_2. Airway-sensitizing effects were noted at lower concentrations, but more information is needed at concentrations that are nearer ambient levels. This study will examine the acute alterations in the guinea pig's histamine responsiveness induced by 1-h exposures to 5 ppm NO_2. Two groups of animals will be exposed: one to room air (n = 10) and one to 5 ppm NO_2 (n = 10). One control and one NO_2 animal will be exposed on each exposure day. Histamine responsiveness will be measured before and immediately after exposure, using methods similar to those described in the previous section.

Pollutant Interaction Studies: Nitrogen Dioxide and Sulfuric Acid

Several epidemiologic studies have suggested a positive correlation between air pollutant levels and frequency of asthmatic attacks. Although H_2SO_4 produces an asthmatic-like response in the guinea pig, it does so only at concentrations that are far above ambient concentrations; the lowest concentration at which we have observed this asthmatic-like response in healthy guinea pigs is 15 mg/m^3. If H_2SO_4 does act to promote airway constrictive responses in human populations, other predisposing factors are probably prerequisite. If airway sensitivity to H_2SO_4 is, like histamine sensitivity, increased by oxidant gas exposures, then NO_2 might act as a predisposing factor. In this study, we will determine if an NO_2 exposure known to produce altered histamine sensitivity in the guinea pig also results in increased airway sensitivity to H_2SO_4. An initial study will use high concentrations of NO_2 and H_2SO_4 to determine whether such an interaction exists. For each of three exposure days, one group of guinea pigs will be exposed for 1 h to 40 ppm NO_2. A second group of animals will be exposed for 1 h to filtered room air. Immediately after exposure, both control and NO_2-exposed animals will be randomly assigned to cages within an H_2SO_4 exposure chamber. Animals will be exposed for 2 h to an H_2SO_4 concentration of 20 mg/m^3 and 1.0 μm in MMAD. We will use excised lung volume as an indicator of airway constrictive response, since our previous experience has indicated that the lungs removed from animals which develop an airway constrictive response are consistently hyperinflated. Animals that die during H_2SO_4 exposure will be removed from the exposure chamber through a pass-through box; their lungs will be excised immediately and lung volume measured by water displacement. Animals that survive exposure will be sacrificed immediately after exposure with an euthanasia solution; the lungs will be excised and lung volume measured. If this preliminary study indicates a positive interaction, studies will be conducted at lower NO_2 and H_2SO_4 concentrations. Combination as well as sequential exposures are of interest and will be examined in subsequent studies.

Pulmonary Effects of Chronic Exposure to Sulfuric Acid, Fly Ash, and Their Combination on Normal and Elastase-Treated Guinea Pigs

This study will examine the response of normal and impaired (elastase-treated) guinea pigs to long-term exposure to H_2SO_4, fly ash, and their combination. The study is designed to model "sensitive" segments of the human population: of various laboratory animals, the guinea pig may possess airway responses most similar to those of the human asthmatic population; elastase treatment will impose an impairment with structural and functional similarities to human emphysema. We plan to conduct lung function and biochemical tests plus morphological evaluations that have been carefully selected to detect subtle pulmonary effects.

Five groups of 60 animals each will be exposed for 6 h/d, 5 d/week, for 3 yr. Of each group of 60 animals, 30 will be treated with porcine pancreatic elastase ~3 weeks prior to exposure. The remaining 30 will be untreated. Present plans are to expose the five groups of animals to (1) filtered room air, (2) low-level H_2SO_4 (in the range of 0.5-1 mg/m^3), (3) high-level H_2SO_4 (in the range of 1-5 mg/m^3), (4) fly ash alone (one level), and (5) high-level H_2SO_4 in combination with fly ash. The H_2SO_4 aerosol will be ~0.4 μm in MMAD. The fly ash will be a well characterized sample obtained from a fluidized bed coal combustor.

Ten normal and 10 elastase-treated animals will be randomly selected from each exposure group. The airway histamine sensitivity of each selected animal will be measured prior to exposure and after 1, 3, and 6 mo and 1, 1.5, 2, 2.5, and 3 yr of exposure. Pulmonary function measurements, including measurements of dynamic compliance and total pulmonary resistance, will be obtained prior to each airway sensitivity measurement. This group of animals will be sacrificed at 3 yr after the start of exposure. Half of the remaining normal and elastase-treated guinea pigs in each exposure group will be sacrificed after 1 and 2 yr of exposure. Other evaluations will be performed on these animals (serially or prior to sacrifice), including measurements of mucociliary clearance and lung function measurements sensitive to small airway dysfunction. The nature and schedule of these evaluations will depend on the outcome of preliminary feasibility studies. At sacrifice, one lung from each animal will be processed for histopathologic and morphometric evaluation. The other lung will be washed for analysis of cytoplasmic and lysosomal enzymes present in the airways. Cytological assays will also be performed on airway fluid samples. The tissue of the washed lung will be analyzed for collagen and elastin content and enzymatic activity.

ACKNOWLEDGMENTS

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REFERENCE

Amdur, M. O., M. Dubriel, and D. A. Creasia. 1978. Respiratory response of guinea pigs to low levels of sulfuric acid. Environ. Res., 15:418-423.

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O. Raabe: Could you give us more details about the chemical and physical characteristics of the fly ash aerosols?

S. A. Silbaugh: The aerosols will come from a fluidized bed combustor at the Morgantown Energy Research facility. If requested, I can provide the details of trace element analysis.

O. Raabe: What is the size distribution, and so on?

S. A. Silbaugh: The size distribution is ~3 μm MMAD, I believe. Maybe Dr. Henderson can comment on that.

R. F. Henderson: The sample we ran previously was 3 μm in MMAD, with a geometric standard deviation of 1.7. In contrast to your fly ash, it's a low temperature, highly porous, and very polydispersed ash.

J. D. Hackney: In the histamine responsiveness studies you used dynamic compliance measurements, correct?

S. A. Silbaugh: Yes.

J. D. Hackney: Did you also look at resistance?

S. A. Silbaugh: Yes, we looked at resistance, but we found (along with the group of Douglas, Dennis, and the late Dr. Bouhuys at Yale) that the best indicator of the airway constrictive response in the histamine-exposed guinea pig is a decrease in dynamic compliance. Resistance changes are more variable.

As soon as the compliance drops by 50%, the animal is removed from the histamine; we don't want any residual effects caused by the spasm itself. All I can say is that the resistance at that time is variable, and we haven't followed it beyond the time at which the compliance drop appears.

P. E. Morrow: You stated that your H_2SO_4 aerosol was 0.4 μm in aerodynamic size. How do you vary the concentration and keep that droplet size constant? Have you done it over the range of 0.1 to 100 mg/m^3?

S. A. Silbaugh: We have a system whereby we can keep the particle size constant. Dr. Wolff has been more involved with that than I have. Dr. Carpenter (Inhalation Toxicology Research Institute) has worked with a system in which dry nitrogen is used to pick up sulfur trioxide, which is then mixed with humid air. Varying the dilutions of the sulfur trioxide and the water vapor as well as the amount of aging permits us to control the particle size of the aerosol.

R. K. Wolff: We have no problem in varying the concentration and keeping the particle size constant. We have generated 0.4-μm particles at concentrations ranging from 100 $\mu g/m^3$ to 100 mg/m^3.

Question: What is the droplet concentration at 100 mg/m^3?

R. K. Wolff: The concentration is ~5 x 10^6 particles/cm^2--the coagulation limit. I'll allude to this a little later.

Question: On all of your function measurements in the guinea pig, were you able to precharacterize your responses (i.e., determine preexposure factors relating to individual animal sensitivity to H_2SO_4)?

S. A. Silbaugh: We did some work with that. In one study, we had two exposure levels that resulted in something like 40/60% responders/nonresponders. We took those two exposure groups and looked for preexposure characteristics that would tend to segregate animals. It does appear that the responsive animals tend to have higher resistance values and lower compliance values prior to exposure. There also appear to be differences in the relationships between, for instance, transpulmonary pressure and resistance. So there do appear to be detectable differences that are related to whether an animal tends to be responsive or nonresponsive.

In an article published a few years ago, Dr. Mary Amdur also reported something like this. Amdur also observed that animals with higher resistance values tended to be more reactive when exposed to various aerosols.

Comment: We're doing H_2SO_4 aerosol studies in humans and we use the sulfur trioxide system of aerosol generation. We've found that [inaudible] and humidity in our chamber determine the particle sizes; we're running 0.1 to 0.3. The concentration we're using is 100 mg/m^3.

In your studies of H_2SO_4 alone, you used 10 to 40 mg/m^3. Have you decided on the concentrations to be used in the fly ash/H_2SO_4 studies?

S. A. Silbaugh: We have not yet decided on the H_2SO_4 concentrations, but we're thinking along the lines of 0.5 to 1 mg/m^3 for the low level and somewhere between 1 and 5 mg/m^3 for the higher level. We can only pick one level for the fly ash; we're thinking of 5 mg/m^3.

Question: Have you permitted the aerosols of acid and fly ash to age together, to examine any possible interactions?

S. A. Silbaugh: No, this would be a preliminary part of the study. The main study would not start until later in the fiscal year, and we have quite a bit of developmental work yet to do. That would be one part of the developmental work.

Question: Regarding the mechanics of the exposure facility, what provisions would be required? Have you any way to pool the two as a single atmosphere, or to provide a means for the aging to occur? Do you think there is any advantage to it?

S. A. Silbaugh: That is not the realm of my expertise. Dr. Wolff, would you care to comment?

R. K. Wolff: It would depend on how much aging you wanted. We could fairly easily go to a few minutes of aging in a relatively large chamber. To go beyond that would involve considerable space and expense. It could be done if it were deemed worthwhile.

Question: What plans have you for varying humidity?

R. K. Wolff: Previously we varied humidity from 40% and 80%. In the planned studies we will probably use 40 to 50% relative humidity. We have not seen striking relative humidity effects. That is another issue we can still address.

M. J. Wiester: The data you showed did not indicate very much of an increase in histamine response up to very high concentrations of NO_2--

S. A. Silbaugh: Are you referring to both NO_2 and H_2SO_4?

M. J. Wiester: I'm not sure.

S. A. Silbaugh: The NO_2 response is quite variable. We're still looking at different ways of analyzing this. For instance, if you consider a particular animal to be an outlier, it may be that a straight line relationship is not the best relationship: it may be that with more animals at higher concentrations we'd see another kind of response. When NO_2 concentrations are expressed as logarithms, the straight line fit does become slightly better.

M. J. Wiester: What I'm saying is that you don't increase your sensitivity of the response at the ambient level--

S. A. Silbaugh: This is what I have outlined for a future study. We need to expose more animals at levels lower than 7 ppm to really get any information regarding that region. The only area where we can say for sure that there is a response is the region of 40 ppm or so. With more animals we should be able to find out.

M. J. Wiester: [inaudible]

S. A. Silbaugh: We found no correlation of H_2SO_4 concentration and histamine sensitivity for the nonresponsive animals. There was a trend at the 2-h point for histamine sensitivity to decrease with increasing H_2SO_4 concentration. Dr. Wolff may be able to shed some light on the implications of these results for future studies. The indications that H_2SO_4 may stimulate mucous production raise the possibility of a mechanism by which increasing H_2SO_4 concentration can actually desensitize animals.

D. E. Gardner: In your NO_2 studies, did some of the animals die?

S. A. Silbaugh: No. We obtained our animals from Charles River in six shipment groups over the course of the study. The shipment groups reported here all responded fairly uniformly. One shipment group (five animals) had no response at concentrations of up to ~120 ppm. We contacted the breeder but could not detect any preshipment differences. Our guinea pigs may actually be less sensitive than rats to NO_2.

M. Goldman: The way the data are plotted on a log scale, aren't you really looking at a two-phase reaction? Anything between 50% and 500% is normal, and then you have a few there--

S. A. Silbaugh: Right.

M. Goldman: Drawing a line with regard to concentrations, it seems to me that you have a uniform response with sensitive animals [inaudible].

S. A. Silbaugh: With NO_2?

M. Goldman: Right. In other words, anything over ~20 gave about the same response.

[Simultaneous discussion]

M. Goldman: If you try to scale it, you pick up the same data linearly and you have what's here.

S. A. Silbaugh: If these points are omitted, I think what you're saying is true.

M. Goldman: I was looking at all of the studies in which you're using this end point. It seems to me that you've just got one group of sensitive animals. The other groups are not sensitive. There's no concentration effect.

T. Crocker: I'm struck by the way in which you're using the relationship of NO_2 to the histamine responsiveness. Heretofore, the histamine response has been used to assist in identifying very low level effects of NO_2; here you're getting a histamine effect and then trying to poison it with a very high level of NO_2. I don't quite understand the rationale for that approach, but it doesn't seem to me to improve our understanding of the responsiveness of the respiratory tract at very limited concentrations.

S. A. Silbaugh: We plan to do those exposures at lower levels. Our histamine challenge procedure is a recent development, the initial purpose of which was to simply quantitate a dose-response relationship for NO_2. We wanted to check our system, you might say. These exposures have served several purposes. First, we did find some information concerning the dose-response relationship. We may be "poisoning it" (as you say) at higher levels, but we did want to see responses in the initial exposures. We planned to go down from there.

We agree that the low concentration range is the important range to study. One thing we did obtain is an indication of a wide variation in individual animal responses. One is thus more encouraged to go back to lower levels and do a large number of animals. We may see no responses in quite a few animals, but there may be animals that are sensitized at very low levels. Starting out with the lower levels might have caused us to become discouraged early, because we wanted to see an effect in this system.

Also, we didn't know what to expect with the H_2SO_4 exposure, and we felt that the NO_2 exposures served as a positive control since others have demonstrated a sensitization to airway constricting agents with exposure to NO_2.

T. Crocker: Yes, but you didn't use NO_2 in the range of 1 to 3 ppm, which is near the limit of that gas' effectiveness in producing sensitization and where histamine makes the measurement of that sensitization more relevant (or at least more detectable, I should say). The issue, really, is that if you go to very high concentrations of NO_2, you may be poisoning the very receptor capability of the system that subsequently also responds to histamine. I'm concerned that we may be overusing the toxic power of NO_2 when what we're really looking for is the ability to identify an increase in sensitivity. [inaudible] NO_2 in the concentrations you're dealing with would be expected to wipe out a large part of the cell population, especially near the alveoli, the [inaudible] of the alveoli junction. NO_2 in these concentrations would also affect cells elsewhere in the respiratory tract, and might very well eliminate, in fact, the animals that respond.

S. A. Silbaugh: I see your point.

D. E. Gardner: I think Dr. Crocker's comments are very valid and pertinent, especially in regard to the discussion yesterday [Chapter 6 of this volume] on the relevance of high doses and how they can perhaps be misleading when you're going to look at low levels of concentration.

26. LUNG CLEARANCE MECHANISMS FOLLOWING INHALATION OF ACID SULFATES

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PROBLEM

Sulfur oxidation products result from the burning of fossil fuels and are widely present in urban atmospheres. Acid sulfates inhaled in the urban atmosphere may have deleterious health effects, and definitive information is needed to set air quality standards. Sulfuric acid (H_2SO_4) mist may be the most irritating of these products, at least with respect to pulmonary function effects (Amdur 1971; Amdur et al. 1975); thus, the study of potential health effects is of considerable interest.

The mucociliary clearance system provides a sensitive indicator of lung injury following exposure to irritant gases and aerosols. Alterations in mucociliary clearance have been observed in man, donkeys, and dogs for H_2SO_4 mist exposures at industrial threshold limit values and below (Leikauf et al. 1979; Schlesinger et al. 1978; Wolff et al. 1978, 1979a, 1979b). Impairments in this important lung defense mechanism may contribute to the development of chronic lung disease or the exacerbation of existing disease. Impairment of mucous clearance might result in increased residence times of toxic materials in the lung, thereby increasing susceptibility to bacterial infection. Inhibition of mucous clearance could also lead to the plugging of smaller airways, followed by atelectasis and a chain of events contributing to chronic obstructive pulmonary disease.

The project reported here explored dose-response relationships between impairment of tracheal mucous clearance and acid sulfate concentrations in acute and chronic exposures of dogs, rats, and guinea pigs. Acute studies were carried out in dogs for reasons of anatomic similarity to humans. Other, smaller animals were used in efforts to identify a chronic animal model that best mimics the human response.

APPROACH

Methods have been developed to measure tracheal mucous clearance in the awake state in dogs, rats, guinea pigs, and rabbits. Microliter quantities of radiolabeled material are instilled in the tracheas of these animals using halothane anesthesia. The animals are allowed to regain consciousness in restrainers and the movement of labeled material is measured using external detection (gamma camera or slit-collimated NaI scanner).

Dogs

Eight beagle dogs were exposed for 1 h to concentrations of 1.0 and 0.5 mg/m^3 H_2SO_4 mist. The particles were 0.9 µm in mass median aerodynamic diameter (MMAD) with a geometric standard deviation (σ_g) of 1.4 as measured by cascade impaction. Temperature was 74°F and relative humidity was 80%. Tracheal mucous velocities were measured 1 week prior to exposure and also 0.5 h, 1 d, and 1 week after exposure. The method was to anesthetize each dog with halothane, insert a fiber-optic bronchoscope into the trachea, turn off the anesthetic, and deposit a 10-µl droplet containing ~20 µCi of ^{99m}Tc macroaggregated albumin (MAA) as the dog started to regain consciousness. Subsequently, gamma camera scintiphotos were taken at 1-min intervals for 25 min in the awake dog to measure the velocity of the labeled material moving up the trachea. Measurements were made of discrete spot velocities and of the velocity of the leading edge of moving activity.

For the 1.0-mg/m^3 exposures, tracheal mucous velocities were significantly depressed after 0.5 h (26% reduction, $p < 0.05$), 1 d (40%, $p < 0.01$), and even after 1 week (30%, $p < 0.05$). Velocities returned to the control range after 5 weeks. Also, a much greater proportion of material remained at the initial deposition site 1 week after exposure than in the control preexposure experiments (47% vs. 14%).

The results for the 0.5-mg/m^3 exposures were somewhat different. Nonsignificant increases in clearance were seen after 0.5 h (35%) and 1 d

(8%). However, a statistically significant depression in clearance was observed 1 week after exposure (35%, $p < 0.05$). These observed abnormalities indicate impairment of an important lung defense mechanism for a prolonged period following acute exposures to relatively low levels of H_2SO_4 mist.

Subsequent exposures of the same 8 dogs to an H_2SO_4 aerosol of smaller size (0.3 µm MMAD) at levels of 1 mg/m^3 and even 5 mg/m^3 showed no significant effects on mucous clearance. Sham experiments were carried out under the same exposure and measurement schedule described above. Velocities were very consistent; there were no significant differences at any time.

Thus, despite the relatively small difference in the size of these two aerosols, a wide disparity in effects was observed. The same pattern was observed in acute toxicity studies using guinea pigs: 0.8-0.9 µm MMAD aerosol was estimated to be 5 times more toxic than 0.3-µm aerosol. The studies reported in this paper indicated a difference of at least a factor of 5, since no effect was observed at 5 mg/m^3 for the 0.3-µm aerosol compared to the decided effects at 1.0 mg/m^3 for the 0.9-µm aerosol.

Our accumulated data indicate that upper airway reflex mediated bronchoconstriction is the predominant mechanism of action of H_2SO_4 mist. Therefore, if more material is deposited in upper airways, a greater effect may be elicited. Deposition studies carried out at the Inhalation Toxicology Research Institute confirm that there is greater upper airway deposition of 0.8-0.9 µm MMAD H_2SO_4 aerosols compared to 0.3-0.4 µm MMAD aerosols.

Rats

Groups of rats were exposed to 1, 50, and 100 mg/m^3 H_2SO_4 for 0.5 h and to 1, 10, and 100 mg/m^3 H_2SO_4 for 6 h.

Trachael clearance was measured in four males and four females in each exposure group 1 week prior to exposure and 1 d, 1 week, and 3 weeks following exposure. (The 3-week measurement was omitted for the 0.5-h exposure.) Rats were anesthetized with 5% halothane to a level of deep anesthesia sufficient to allow intratracheal instillation of ^{99m}Tc MAA. The rats were placed in plastic restraining tubes, allowed to regain consciousness, and driven past a slit-collimated NaI detector to produce a profile scan of the labeled material in the trachea. Profile scans were taken at 0, 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, and 60 min after initial instillation. The percentage of activity remaining at the instillation site after 1 h was the principal measure of clearance. Tracheal mucous velocities were also determined by measuring the movement of the leading edge of material. The two types of measurements correlated well but the retention measurements were more consistent. Two-tailed paired t-tests were used to detect statistically significant changes in clearance.

Speeding in clearance was observed following the 0.5-h exposure. No changes were observed for the control group, but dose-related changes were observed for the exposed groups. Significant speeding of clearance was observed after both 1 d and 1 week following the 100-mg/m^3 exposure. For the

50-mg/m^3 exposure, a slight depression of clearance was observed at 1 d, while moderate speeding was observed at 1 week. For the 10-mg/m^3 exposure, a speeding in clearance was observed only at 1 week after exposure.

Following 6-h exposures, dose-related speeding in clearance was again observed, with the most striking results at the highest exposure level (100 mg/m^3). Clearance was enhanced for the 100-mg/m^3 exposure at 1 and 3 weeks following exposure; for the 10-mg/m^3 exposure at 1 d; and for the 1-mg/m^3 exposure at 3 weeks. No significant changes were seen in the control group, and clearance results were highly reproducible. Clearance effects were consistent for the two sexes except at 1 d after the 100-mg/m^3 exposure. At that time speeding was observed in females but not in males.

Biochemical response in the lavage fluid and in the lung tissue was minimal. In lavage fluid, the only detectable response to any level of exposure at 1 d was an elevation in sialic acid, a marker for acid mucous glycoproteins. As for clearance, this elevation occurred at the 100-mg/m^3 level in females only. Sialic acid levels were always increased either preceding or in coincidence with increases in mucous clearance. Scanning electron micrographs also indicated increased mucous secretions in the trachea.

Speeding in mucous clearance has been observed in dogs, man, and donkeys but only during or soon after low-level exposure (<1 mg/m^3) (Leikauf et al. 1979; Schlesinger et al. 1978; Wolff et al. 1978, 1979a, 1979b). The speeding in clearance that was observed in rats after both 0.5- and 6-h exposures to H_2SO_4 mist occurred at levels of up to 100 mg/m^3--100 times the industrial threshold limit value. This speeding in clearance was somewhat surprising, and the reasons for it are not clear. Figure 26-1 conceptualizes the observed disparities between the rat and the other extensively studied species. Speeding was observed in rats at all levels; in the other species, however, clearance was generally increased at low levels but depressed at higher levels (>1 mg/m^3). These results are quoted for aerosols in a similar size range (0.5-0.9 μm MMAD).

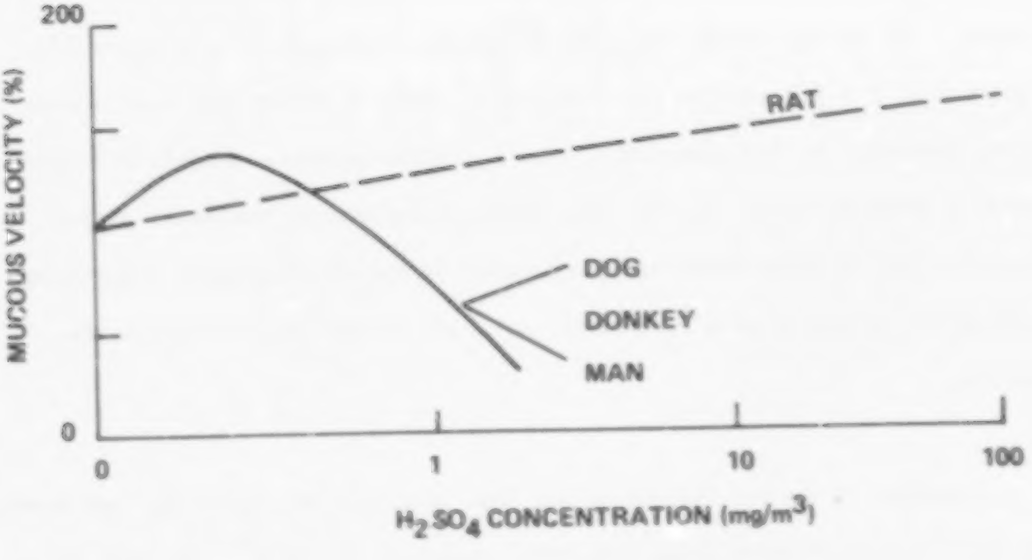

Figure 26-1. Mucous velocity by species following exposure to H_2SO_4.

PLANNED RESEARCH

Results from the rat model do not appear to agree well with results from human or dog experiments. Therefore, the rat may be a poor model for chronic exposure studies. However, the guinea pig has been demonstrated to show pulmonary function responses to H_2SO_4 similar to those of humans. Accordingly, we have initiated mucous clearance studies with guinea pigs. Recently obtained base-line data show mucous clearance patterns similar to those in rats and rabbits. In these studies, tracheostomies were used to deposit the labeled markers. We are starting to investigate clearance in guinea pigs using instillation via the intratracheal route. This has been difficult because of the guinea pig's sensitivity to laryngospasm. However, this problem has been solved and studies are about to begin. The advantage of intratracheal instillation is that animals can be used as their own controls (as demonstrated in the previous dog and rat studies).

Acute Exposures

For guinea pigs, we plan 1-h exposures to H_2SO_4 at levels of <1 mg/m^3 with particle sizes of 0.3-0.4 µm and 0.8-0.9 µm MMAD. Following completion of these studies, we plan 1-h exposures to $(NH_4)_2SO_4$ and NH_4HSO_4 at levels of <1 mg/m^3 and particle sizes of 0.3-0.4 µm MMAD. Also, 1-h exposures to 5 mg/m^3 fly ash and 5 mg/m^3 fly ash + 1 mg/m^3 H_2SO_4 will be carried out. These studies will be a prelude to chronic exposures. For dogs, we plan 1-h exposures to $(NH_4)_2SO_4$ and NH_4HSO_4 at <1 mg/m^3.

For all acute exposures, if no effects are initially noted at 1 mg/m^3, we will conduct more extended or repeated exposures at this level.

Chronic Exposures

We plan chronic exposures to H_2SO_4 and H_2SO_4 + fly ash according to the schedule outlined under project RPIS Number 4054, Acute-Chronic Loss of Lung Function Following Inhalation of Acid Sulfates. Mucociliary clearance measurements performed on the same schedule will serve as pulmonary function measurements in normal and elastase-treated guinea pigs.

ACKNOWLEDGMENTS

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WORKSHOP COMMENTARY

Comment: You examined the clearance of particles over a fairly short period. In the first phase of clearance, you did have some residual particles; of course, these disappear radioactively. Is anybody looking at the long-term clearance of residual particles?

R. K. Wolff: Currently, a number of studies at the Inhalation Toxicology Research Institute are investigating long-term clearance of particles. We may do some longer-term clearance studies in connection with those outlined here.

27. OVERVIEW OF CURRENT AND PLANNED RESEARCH BY THE INHALATION TOXICOLOGY BRANCH

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INTRODUCTION

This report presents an overview of current and projected research on oxidants by the Inhalation Toxicology Branch (Environmental Toxicology Division, Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina). Elsewhere in this volume, Graham (Chapter 28) and Miller (Chapter 29) discuss some of these studies in greater detail.

STAFF, FACILITIES, AND BUDGET

The Inhalation Toxicology Branch (ITB) is staffed by ~22 permanent, full-time employees and ~13 part-time employees. The Branch is divided into three sections: Pharmacology and Microbiology, Physiology, and Inhalation Exposure and Dosimetry.

Each Section employs individuals with a wide variety of scientific backgrounds. For example, in the Microbiology and Pharmacology Section there are several microbiologists, pharmacologists, and a number of biologists; there are also entomologists who are working in the area of unconventional or biological pesticides (i.e., using viruses, bacteria, and protozoans as pesticides). The Physiology Section includes individuals with expertise in cardiovascular disease, physiology, biochemistry, and organic chemistry. The Inhalation Exposure Section is composed of inhalation engineers, electronic technicians, physical scientists, and a biostatistician.

In addition to these individuals who are directly employed by ITB, our program is supported by ~22 in-house contractor personnel from Northrop Services, Inc. - Environmental Sciences. The majority of these individuals provide direct support for the inhalation exposure and engineering needs of the Branch. They are responsible for maintaining the exposure facilities, which includes generating and monitoring a large number of chemicals of interest to EPA. Northrop also maintains individuals trained in the areas of physiology, microbiology, and pharmacology who perform research in certain areas of our base program.

Comparing the allocation of funds for 1979 and 1980 indicates how our research program can shift from one year to the next. In fiscal year (FY) 1979, 41% of our total budget was devoted to the energy program, whereas in FY 1980 there was no money to either continue or start any studies in that area. In FY 1980 there was a significant increase (5% to 21%) in criteria research funding, and a large jump (19% to 51%) in non-criteria pollutant research. We are also involved in one transportation program whose objective is to compare the carcinogenicity of diesel exhaust and various diesel extracts with roofing tar, cigarette smoke condensate, and coke oven emissions. This work is being done under a grant and represents ~15% of our total budget. There is also a small program (5%) in pesticides and toxic substances.

For FY 1980, ~35% of our total budget is allocated to in-house programs; the balance (~65%) is funded extramurally under grant programs, cooperative agreements, or contracts.

GOALS FOR CRITERIA POLLUTANT RESEARCH

In order to make our research most useful for the setting of criteria, we incorporate certain broad objectives in our research planning. First, we're interested in making animal data more relevant to man. Others have stressed that "a mouse is not a man" (e.g.: Chapters 4, 5, and 7 of this volume). What we want and need to do is to develop a scientifically sound data base that can be used to develop modeling systems that will help us extrapolate animal data to humans. For example: If a mouse receives a certain concentration of a pollutant gas at its nose and subsequently shows a certain health effect, what concentration would have to be at a man's nose to show a similar effect, assuming the same kinds of target organs or target tissues were affected? Such information would greatly improve the usefulness of animal toxicology data.

Secondly, we are also interested in confirming and understanding many of the important toxicological studies that presently comprise the data base for establishing a scientifically sound standard for ozone (O_3) and nitrogen dioxide (NO_2) and other criteria pollutants.

Our program is interested in interactions. It's time to get away from studying single pollutants. Other chapters in this volume demonstrate that there are already many studies examining the effects of such combinations as O_3 with NO_2; sulfuric acid (H_2SO_4) with O_3; and H_2SO_4 with NO_2. As time goes on, we will expand these mixture studies to include other gaseous and particulate pollutants (such as hydrocarbons, sulfur dioxide, etc.).

It is imperative that we continue to identify any new effects or end points having potential utility in human studies. For example, during the past few years, we have established a very comprehensive program specifically devoted to the study and development of new models for measuring in small animals various sensitive pulmonary functional parameters similar to those measured in humans. Our program continues to seek noninvasive techniques that will hopefully provide indicator systems useful to both the epidemiologists and to the clinical investigators.

Lastly, we are interested in developing new animal models that mimic the special human subpopulation that is most sensitive to environmental chemicals. A number of new animal models of human diseases are now available for application by toxicologists in environmental studies.

POLLUTANTS STUDIED

To date we have investigated the effects of exposure to O_3, NO_2, and combinations of O_3 with NO_2, with particulates, etc. Some work has been conducted under contract to determine the health effects of exposure to peroxyacetyl nitrate. We continue to study the effects of complex mixtures, including the combination of O_3, SO_2, and trans-2-butene. Our research program also considers pollutants that are beyond the scope of this volume (e.g., pesticides, toxic substances, non-criteria pollutants).

MODEL SYSTEMS IN USE

Biological indicators of health effects include both interaction with infectious microorganisms (e.g., bacteria, virus, Mycoplasma) as well as specific alterations in a number of specific host defenses. Included here are functional and morphological alterations in alveolar macrophages, various humoral and cell-mediated immunities (including B and T cell transformation), and antibody titers.

Other studies investigate the effects of various environmental chemicals on upper respiratory clearance mechanisms. In this model system we examine changes in ciliary function by measuring the beating rate and frequency, and we attempt to relate these changes to histological abnormalities of the cilia.

J. O'Neil, M. J. Wiester, and J. A. Raub of our Branch have developed a battery of pulmonary functional tests that should be very useful in measuring various changes in respiratory rate and other functional parameters in small animals.

With O_3 and NO_2, current studies model the pulmonary deposition and the fate and absorption of these gases in the lung. Although we are primarily interested in the lung as the target site, we investigate other organ and target tissues for any extrapulmonary effects that might result from inhaling these environmental chemicals.

Animal models are employed to measure cardiovascular effects of O_3, NO_2, and other compounds of interest to EPA. The end points include identification of lipid profiles, EKG measurements, and determination of other physiological parameters.

Another model focuses on pentobarbital-induced sleeping time. Early results indicate a significant sex difference: females are much more susceptible to the actions of NO_2 and O_3 than males. Graham (Chapter 28 of this volume) expands on these studies.

When indicated, we complement all our studies with examination of various lung and serum biochemical end points and/or performance of necessary histopathology.

In addition to our in-house research efforts, we are collaborating with investigators at the University of North Carolina in a study of how the microorganism Mycoplasma pneumoniae interacts with normal systems and of how airborne pollutants may alter the microbial action upon the upper airway. This model system is discussed further in the following section.

MODEL SYSTEMS UNDER DEVELOPMENT

Several model systems are presently under development. In our acute infectivity system, bacteria are given after exposure to a gaseous pollutant. In a normal animal or an animal that is not affected by the pollutant, the bacteria are all rapidly removed or killed within 6-8 h; the animal's lungs become normal again (i.e., free of the inhaled bacteria). This very sensitive model is excellent for mimicing acute infectious diseases but fails to resemble a chronic infectious disease.

A chronic respiratory model is being developed by E. Hu of our Branch. This model uses the bacterium Mycoplasma pneumoniae. In these studies, the animals exhibit a low-grade infection which mimics chronic bronchitis. We plan to soon examine the effects of various pollutants using this chronic respiratory disease model.

A viral model system similar to our acute bacterial infectivity system but employing viruses instead of bacteria is being developed by M. J. Selgrade of our Branch. This project investigates the effects of environmental chemicals on the reactivation of latent viruses and the interaction of viruses and bacteria.

There is also interest in better defining the susceptible human subpopulation. One study at Duke University will attempt to determine the effects of pollutants on the young developing lung. This study will resemble the work by Dungworth (Chapter 20 of this volume) but will involve exposures to NO_2 and to O_3 for longer periods of time in hope of correlating the morphological effects with various pulmonary functional changes. Under this cooperative agreement study, we plan to perform detailed morphometrics as well as identification of various biochemical changes in the lung.

There is also a project whose objective is to examine individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency and to determine how they respond to O_3 assault. The investigators have bred G6PD-deficient mice and sheep and are attempting to find out if they are more susceptible to oxidants than normal animals.

An animal model of emphysema is being developed. Animals which are exposed to elastase develop emphysema-like lesions; our program is attempting to correlate and quantitate the amount of emphysema using pulmonary functional techniques. Elastase-exposed animals will be exposed to various pollutants and their responses compared to proper controls. This model mimics an individual in the population who has emphysema and is exposed to a pollutant.

To complement this study, we plan to breed and expose a strain of mouse (the blotchy mouse) that is genetically prone to develop emphysema.

Finally, there is interest in how diet deficiency may alter susceptibility to O_3 and to NO_2. The biological end points include various barrier functions, the production of edema, the breaking of tight junctions in the upper airway, and similar biochemical and morphological indicators of damage.

STUDIES TO BE REPLICATED

There are some important studies that we plan to replicate and/or extend. One of these is a study by Bartlett et al. (1974) which examined the effects of oxidant pollutant (0.2 ppm) on the young developing lung. These authors concluded that the developing lung is more susceptible to O_3 than the adult lung.

Using a very sensitive model, Sherwin (Chapter 36 of this volume) detected edema in the lung at a very low level (0.4 ppm) of NO_2. We plan to extend and replicate this study.

Some years ago, O_3 was found to accelerate aging (Stokinger 1957). There is a need to repeat this study to better define those results. Unfortunately, the funds are not available for FY 1980.

Finally, it was recently reported that exposure to 0.2 ppm NO_2 causes a decrease in metabolism of prostaglandin E (Menzel et al. 1976; Menzel 1980). These results also demand replication to better define the mechanism of action and possible consequences.

All of these animal studies represent important components of the existing data base (and therefore of the criteria document). For that reason, they have high priority for funding.

COLLABORATION WITH OTHER INVESTIGATORS

In addition to our in-house efforts, we participate in a considerable amount of collaborative work with other investigators in the Research Triangle area. Within EPA's Health Effects Research Laboratory, we have joined the Experimental Biology Division (N. Chernoff) in a search for possible teratogenic effects of NO_2. A similar collaboration (with L. Reiter) seeks to demonstrate behavioral effects of exposure to O_3 and NO_2. With S. Sandhu (Genetic Toxicology Division) we are searching for mutagenic effects of NO_2 exposure.

There is also collaboration with the University of North Carolina, Duke University, and North Carolina State University. Topics of these studies include immunologic effects of O_3 and NO_2, effects of oxidants on upper respiratory tissue (scanning electron microscopy), and pharmacokinetic effects of O_3 on some xenobiotic agents.

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28. SOME SPECIFIC STUDIES PLANNED BY THE INHALATION TOXICOLOGY BRANCH

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INTRODUCTION

This report discusses in more detail some of the projects overviewed by Gardner (Chapter 27 of this volume). All are projects of the Inhalation Toxicology Branch (Environmental Toxicology Division, Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina). It is not the intent of this report to present data, but rather to indicate the focus and scope of these studies.

CHRONIC NITROGEN DIOXIDE EXPOSURE USING MOUSE INFECTIVITY MODEL

One major planned study is a chronic nitrogen dioxide (NO_2) exposure. We recently built an in-house exposure facility suitable for NO_2 (or any gas). Currently, we are conducting some shorter-term studies to ensure that the system will work when we actualy begin the chronic exposure.

The driving force behind this study is some previous work with the mouse infectivity model. In this model system, a mouse is exposed to a pollutant and then challenged with an aerosol of live Streptococcus pyogenes. Mortality is followed for a holding period in clean air. This model system is one of the most sensitive model systems available for ozone (O_3) and NO_2 animal inhalation toxicology.

Previous NO_2 studies examined concentrations no smaller than 0.5 ppm. At 0.5 ppm, 90 d of exposure were required to observe effects. We are, of course, interested in investigating concentrations that are closer to ambient levels. Therefore, it will be necessary to extend the time of exposure. Prior work also showed that, at least for NO_2 and the infectivity model, accurate interpretation of results requires that the chamber exposure patterns match ambient exposure patterns. Therefore, we will perform more research using a base-line NO_2 concentration upon which peak NO_2 concentrations are superimposed. In the planned study, we will expose the animals to 0.2 ppm continuously (except for short periods of cleaning the chamber). Then the animals will receive a peak exposure of 0.8 ppm, 5 d/week, 1 h/d in the morning and 1 h/d in the afternoon.

A number of parameters will be examined. Besides the Streptococcal infectivity model, several mouse pulmonary function tests are now available and will be applied.

PENTOBARBITAL-INDUCED SLEEPING TIME IN THE MOUSE

Although over the years it has been assumed that gases affect primarily the lung, there is increasing interest in the extrapulmonary effects of gases and other pollutants. There have been recent indications that extrapulmonary effects occur upon exposure to oxidizing pollutants. One of the model systems used in our laboratory is pentobarbital-induced sleeping time in the mouse. In this particular model system, the animal is exposed to a pollutant, pentobarbital is injected, and sleeping time is measured.

In our early studies, concentrations as low as 0.1 ppm O_3 and 0.25 ppm NO_2 caused significant increases in pentobarbital-induced sleeping time. Further evaluation of this model led to some interesting results. For example, there appeared to be a difference between the toxicity of NO_2 and O_3. As the concentration of O_3 was decreased (e.g., from 1 ppm to 0.1 ppm), an increasing number of days exposure (at 3 h/d) was required to observe increased pentobarbital-induced sleeping time. A single 3-h exposure to 1 ppm O_3 produced the effect, whereas at 0.1 ppm it was necessary to repeat the exposure (3 h/d) for 16 d to observe about the same level of effect. However, with NO_2 the effect could be seen after the first day of exposure: a 3-h exposure to 5 ppm NO_2 or to 0.5 ppm NO_2 demonstrated the effect. Thus, in this model system (at least for acute exposures) NO_2 appears to be more toxic than O_3. Of course, this conclusion differs from the vast body of toxicological literature on the two gases. Our results suggest a different mechanism of action between O_3 and NO_2, and this is something that we want to follow up.

To make sure that our results were not unique to pentobarbital, further examinations employed a variety of drugs (hexobarbital, thiopental, and zoxazolamine). We found that our results were indeed not unique to pentobarbital.

We were also interested to determine the biological significance of these effects. With regard to criteria documents, the concentration is important but the effect is of prime importance. Knowledge of the significance of that effect (e.g.: Are human beings at risk if this effect is observed in animals, or even in humans?) is a very, very important question. This model system is particularly useful because effects are observed at lower concentrations.

Disappearance curves for pentobarbital in the blood were performed to determine (1) if there were effects on either phase 1 or phase 2 clearance, and (2) if the effects were primarily on hepatic metabolism. Generally speaking, changes in pentobarbital-induced sleeping time were attributed to quantitative changes in xenobiotic metabolism.

Once we complete the kinetic studies described above, we plan to employ another model system has already been developed. This model system, antipyrine kinetics, is based on the fact that antipyrine is completely metabolized by the cytochrome P-450 system. In humans, antipyrine is an indicator of P-450 metabolism by the liver. We plan to test this model in animals and perhaps use the results to provide some guidance to workers engaged in human studies. Perhaps similar studies can eventually be performed in humans and then related to the whole array of effects that can be observed using the more invasive techniques with animals. Besides looking at some of these whole-animal models, we want to examine in more detail some of the events that may be occurring in the liver with the cytochrome P-450 system. Thus, we will also run a rather typical evaluation of enzyme activities (O-demethylase, N-demethylase, and hydroxylase).

Sex sensitivity studies were part of our initial evaluation of this model system. (Given that EPA is charged with protecting the most sensitive segment of the population, sex sensitivity is an appropriate focus of research.) We began with female mice and the effect was observed. Then came the question: Is there a sex sensitivity, or does this effect only occur in the mouse? (An effect that occurs only in the mouse might not be extrapolatable to man.) We found that the effect occurs in the rat and hamster as well as the mouse; in all these species, the female was more susceptible. There are known sex differences in P-450 metabolism in the rat, but these major differences do not apply to other species. Thus, the sex difference we observed represents more than classical differences in P-450 metabolism.

SENSITIVITY TO BRONCHOCONSTRICTING AGENTS

Our program includes a large battery of studies within the category of lung metabolism and pharmacological regulation of the lung. The major stimulus behind this body of research is the increase in airway resistance observed in humans after exposure to O_3 and NO_2. This particular effect in humans is one of the major reasons behind regulation of these pollutants, and we want to better understand its mechanism of action.

Many current human clinical studies involve pharmacological perturbation with carbachol, acetylcholine, or other parasympathomimetic agents to see if the pollutant increases sensitivity to these bronchoconstrictors. Questions have been raised as to the biological relevance of increased sensitivity to bronchoconstrictors and as to how EPA should treat this information from a regulatory standpoint. Our group is conducting some mechanistic studies that are designed to yield an improved understanding of the toxicological events, thereby giving a better understanding of the potential seriousness of these events in man.

We are conducting a number of studies with O_3 alone and with NO_2 alone. After we define the effects with these gases, we plan to expose the animals to the same gases in combination. Early studies by Menzel (see Gardner, Chapter 27 of this volume) focused on NO_2 effects on the metabolism of prostaglandins, particularly PGE_2. Concentrations of NO_2 as low as 0.2 ppm (3 h) inhibited metabolism of the bronchoconstrictor; the effect was maximal at 18 h post exposure (i.e., there was a delayed effect). The study used an isolated ventilated perfused lung (IVPL), so the effect observed was probably on the epithelial cells of the capillaries in the IVPL preparation. We are interested in expanding this study because the prostaglandins affect not only airway tone but also vessel tone, and changes in these parameters might influence not only airway resistance but also the relationship between ventilation and perfusion in the lung. Also, there are a number of other possible effects. Hopefully, expanding this study will lead to a better understanding of the mechanism involved in airway resistance changes.

Other compounds (e.g., histamine and SRS-A) are also very involved in bronchoconstriction, and we plan to measure those compounds as well. All are related to arachidonic acid metabolism and products from the associated reactions.

Also, the lung is the main site of the converting enzyme activity that takes angiotensin 1 to angiotensin 2 (the most potent vasoconstrictor in the body). We have accomplished some preliminary studies indicating possible effects on these systems. We plan to extend these studies and see if changes in the reflex system will be reflected in changes in cardiovascular function.

Planned studies that have not yet begun include an examination of cyclic AMP and cyclic GNP; these are very important regulators of various aspects of cell function. Also planned are studies of antioxidant metabolism. Most of this work will be done by M. Mustafa (University of California - Los Angeles).

Many of Mustafa's studies will examine combinations of O_3 and NO_2, something in which we are particularly interested in light of his previous reports of effects at concentrations as low as 0.1 ppm (and also at low concentrations of NO_2). We need to know more about combinations of effects and recovery from those effects.

STUDIES TO IDENTIFY SUSCEPTIBLE POPULATIONS

With regard to what segments of the human population are more susceptible and might need more protection, there are some obvious lines to be followed: male vs. female, very young vs. young vs. older, and so on. These are very large classifications. In spite of many years of toxicological studies with O_3 and NO_2, it still remains unclear as to whether the young are more or less susceptible. Our group wants to improve the state of this knowledge; so far, the only solid plans are for O_3. However, there is a need for such work with NO_2, also.

The planned study will use rats. Animals will be exposed to 0.25 ppm, 0.12 ppm, and 0.08 ppm O_3. A set of animals will be exposed from 0 to 6 weeks of age (when alveolarization occurs). Also, a group of young adult animals will be exposed. A control group of young adults will be exposed to O_3 as well as air.

Morphology and extensive morphometric procedures will be the prime parameters used to evaluate age susceptibility. Pulmonary function will be inserted into the system as soon as the animals are large enough to be measured. It should be possible to do a reasonable battery of pulmonary function studies when the animals reach ~20 g.

WORKSHOP COMMENTARY

R. K. Wolff: Did you mention that you were going to use O_3, NO_2, and some inhaled particulate?

J. A. Graham: As part of our Branch's inhaled particle program, we're interested in doing studies on the combined effects of particles plus gases. We have not yet decided precisely what particles; the decision will be based upon data from ongoing studies.

J. L. Whittenberger: The theme of this meeting is scientific research; a secondary theme is relationship to standard-setting. Gardner [Chapter 27 of this volume] gave as one of the major objectives of your Branch the confirmation and extension of important findings. I agree that that's a very important objective. It was mentioned that you'd like to confirm and extend the Bartlett et al. (1974) study [see Gardner, Chapter 27 of this volume, for details of reference]. The importance of that study was recognized in 1977, if not earlier. Would you be willing to give a mini case history of why the Bartlett study has not yet been confirmed and extended?

D. E. Gardner: My guess is that it has mainly been a matter of appropriate funds and priority. In reviewing the documents, it becomes evident that this is an important study when one considers the whole package of animal toxicology programs. Now that we have some very sophisticated morphometric techniques, we think we can not only reproduce the study but also extend it and make it better.

J. L. Whittenberger: EPA didn't have to do that study with its own funds; there's NIEHS "just down the road." Why didn't they repeat it?

J. A. Graham: Essentially it comes down to funds and also to the study's relationship to criteria documents. When a criteria document is evaluated by a large number of health scientists, the gaps really become immediate. That's why it's a good exercise to read the criteria documents even if you're not involved in the regulatory process: you see where the research is needed.

J. L. Whittenberger: When you say it's a matter of "funds," are you telling me that somebody in Washington screwed up?

D. E. Gardner: We hope to correct this.

J. A. Graham: Dr. Gardner [Chapter 27 of this volume] discussed the relatively low level of funding for oxidants research in fiscal year 1979 as compared to fiscal year 1980. There is a major difference.

D. E. Gardner: If we had the money to fund the projects of every researcher attending this workshop, all those projects would be very interesting. But there is a limited budget, and with this limited budget we have to answer to "the guy that's paying the bill." That's why we can only fund projects that the customer says are most useful in the regulatory setting.

J. L. Whittenberger: So you're saying that the Program Office doesn't consider this particular study to be very important?

J. A. Graham: No, indeed they do consider it to be important. There was simply a financial limitation last year. I'm sure everybody in this room could propose 10 excellent studies to add on to those outlined here by Dr. Gardner and me. But we simply can't do it all at once. You're going to be asking the same question about a different study four or five years from now.

D. E. Gardner: Part of the objective of this workshop plus the "straw man" document [Miller et al. 1979; see Chapter 2 of this volume for details of reference] is to bring some of these elements together. For example, Dr. Dungworth's work [Chapter 20 of this volume] will fit in very nicely. Maybe bringing all of these together will help solve some of our problems.

J. L. Whittenberger: Remember that 88% of environmental health research is not funded by EPA.

Question: Can you provide a better explanation of how the decisions were made? In answer to Dr. Whittenberger, you pointed out that one pressure on you is the necessity to respond to your client. What client is interested in the mechanistic work you were talking about; what client considers that top priority?

J. A. Graham: The Oxidant Research Committee of the Office of Research and Development.

D. E. Gardner: As described by Jones [Chapter 4 of this volume], all of our work goes through research committees made up of clinicians, epidemiologists, chemists, OAQPS staff, lawyers from the Office of General Counsel, and so on. Every time we have a package that we want to "sell," we present it to these committees. The committees then prioritize it. To the best of our ability, we present what we think is appropriate.

Question: [inaudible]

J. A. Graham: Are you asking if a study done in rats can be extrapolated to humans? I think Miller [Chapter 29 of this volume] addresses the quantitative aspects of that question. As a generalization, we feel that if an effect can be demonstrated in a number of animal species then it is quite possible that a similar type of effect occurs in humans. We don't know yet at what concentration that effect would occur in humans. But if young rats are more sensitive than middle-aged rats, it's quite possible that a similar effect occurs in humans. The results of such a study would provide guidance for future epidemiologic studies or even human clinical studies (if they were ethically possible).

Comment: How can one use cyclical exposures (especially with a base-line exposure underneath that) as information in the standard-setting process?

J. A. Graham: EPA has two major information needs with respect to NO_2; these relate, respectively, to a short-term standard and a long-term standard. Should each exist and, if so, at what concentration? The prime drive behind this study is to answer questions relating to the short-term standard.

At present, EPA has an annual standard for NO_2 which permits the occurrence of cyclical peaks. The question is: Are cyclical peaks safe for the public health, or do these commonly occurring peaks add an additional burden for which control measures are needed? Our planned study will expose animals to these patterns. One control will be the base-line concentration of NO_2 itself. Then we will have base line plus peak. Let's say that we add an incremental 20-30% effect to the system. That might also apply to humans. The ratio we have chosen for this particular chronic study (0.2:0.8) is the typical urban ratio that occurs in the ambient environment.

D. E. Gardner: Those experimental concentrations may be slightly higher than concentrations in the natural environment, but the ratio is consistent.

Question: When you go about setting a standard, then, you need some of the information to help you set long-term standards and some for short-term standards?

D. E. Gardner: Yes.

Comment: In several of the studies [described in this volume], the rat appears to be an outlier, both with respect to other rodents and with respect to other species. Why are you beginning another rat study?

J. A. Graham: The age susceptibility study will be done in rats because the Bartlett et al. (1974) study was done in rats. We want to replicate and extend that work. Also, a lot of base-line morphometric information is available for rats, and it will probably be possible to share some controls with other studies.

I think the whole question of species sensitivity to O_3 and NO_2 (or to any pollutant, for that matter) is a very difficult issue. At this time, in terms of pulmonary effects, I don't know which species is more or less sensitive. At this workshop we heard an excellent presentation [Chapter 11 of this volume] on some of the differences that exist, let's say, in circulating anti-oxidant enzymes.

I think the rat has been used by Mustafa and Freeman. In terms of the lung, Mustafa found effects at 0.1 ppm O_3 for rats maintained on normal Vitamin E levels. For rats with excess Vitamin E levels, the effect was not seen until 0.2 ppm. So I think the rat is sensitive, at least in terms of lung effect, and lung effects are primarily what we will examine in this study.

Once again it's a question of limited funds. I agree that it would be ideal to follow up these effects in several animal species.

29. BIOMATHEMATICAL MODELING OF OXIDANT TOXICITY

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INTRODUCTION

There is an acknowledged need to better assess target organ exposure levels and the comparability of the animal toxicological data base for standard-setting purposes. The current standards for oxidant gases are based mainly upon human studies. All of the animal toxicological data (which are 95% of the data available) come in "through the back door": "These effects are observed in humans and, by the way, some effects are also observed in animals." In ethical consideration of what cannot be done experimentally with humans, and in view of the need to understand mechanisms, researchers must provide answers that will allow EPA to make better use of the animal toxicological data base.

The problem is partially one of dosimetry: How do we correlate an effect observed in an animal toxicological study with the exposure concentration required to deliver the same amount of pollutant to the target site in man?

We must improve our determinations of the quantities of pollutants that reach given levels in the respiratory tract and that are associated with given effects.

This report describes some preliminary attempts to biomathematically model the deposition of ozone (O_3). Biomathematical modeling of deposition is an expanding field, and one of increasing interest to EPA. At present, the technique is not utilized to the greatest possible extent. Fuller utilization will require more and better biochemical and physical data. Thus, another goal of this report is to outline some areas in need of further research. Many of the needed studies are not specific to O_3, but would apply to modeling the deposition of any gas.

NASAL PHARYNGEAL REMOVAL

It is exceedingly difficult if not impossible to measure the specific amount of pollutant that reaches a given level in the lung. It is certainly possible, however, to experimentally measure nasal pharyngeal removal. Nasal pharyngeal removal of O_3 in humans represents a major piece of missing data that would enable us to put into perspective the animal studies and the effects observed in human exposures. This information would permit various manipulations to relate the likelihood of exposure relationships to probability of effect.

In one particular study (Miller et al. 1979) we looked at nasal pharyngeal removal in rabbits. This was also done in guinea pigs. Both species showed 50% removal of the pollutant over a concentration range of 0-2 ppm. These results for O_3 satisfy such other models as that of Aharonson et al. (1974) for penetration of soluble vapors into the nasal mucosa. In guinea pigs, if the concentration exceeded 2 ppm, there was disproportionate removal by the nasal pharyngeal cavity (an increase of 15%) and the similarity of deposition was no longer maintained.

GAS TRANSPORT

The major components for analyzing gas transport are (1) convection, which can be separated into advection and eddy dispersion, and (2) diffusion in both the axial and radial directions, which is a function of the flows in various areas in the lung. To accurately model the amount of pollutant removed at each level of the lung also requires the incorporation of any chemical reactions in addition to morphometric data. Fortunately, the morphometric data are starting to become available.

MODELING PROCESS

These major components are brought together in a differential equation that represents gas transport in the lung. The change in concentration per unit time is a function of convection, axial and radial diffusion, and the source terms. For a given pollutant, then, one needs to know these values in order to definitively solve the equation. As discussed below, we have completed preliminary modeling of O_3. However, much work remains to be done to improve the data base.

MODELING ABSOLUTE DOSAGE: SOME PRELIMINARY RESULTS

In our initial O_3 modeling, we used a reaction scheme that looks at the attack of O_3 on free fatty acid carbon-carbon double bonds. Also incorporated were Mudd et al. (1969) data showing the reactions of O_3 with the free amino acids. Available data on lipids obtained from human tracheobronchial secretions were also used.

After this initial work, we found data on the free amino acid concentrations in tracheobronchial secretions (Köhler et al. 1969). The results discussed below were obtained in updated modeling which incorporated these new data. This illustrates that we can always refine our estimates of dose by incorporating additional biochemical data.

With respect to dose for each airway generation in the human lung, the model predicts that the seventeenth generation--the first generation of respiratory bronchioles--will receive the maximum dose of O_3. For low concentrations, the model predicts no penetration of O_3 through the mucous layer to conducting airway tissue; as the concentration increases, penetration into these other generations occurs. However, for a given concentration it is still the respiratory bronchioles that are hardest hit. Proceeding distally, the predicted dose decreases. In terms of the available histopathological data for primates and rats (Dungworth et al. 1975; Stephens et al. 1973), these results correlate well for main site of injury.

We also obtained modeling results for rabbits and guinea pigs. The available morphometric data for these species are not exactly comparable to humans, but there are similarities in terms of tracheobronchial bronchioles, alveolar ducts, sacs, etc. The junction between the last bronchioles and the alveolar ducts is again hardest hit in terms of O_3 dose. Also, the conducting airway dose again falls off as one proceeds distally. The shapes of the curves are quite similar; there are differences in the absolute magnitude of the predicted dose.

In guinea pigs, higher concentrations are required to predict dosages to the conducting airway tissue. Once again, the terminal bronchiole, alveolar duct, and respiratory bronchial areas are predicted to receive the maximum dose.

We plotted maximal dose as a function of inhaled tracheal concentration for man, rabbits, and guinea pigs. In our first plots, these curves started to become linear above 100 $\mu g/m^3$. When we incorporated the new data on tracheobronchial free amino acids that are available to react with and deplete O_3 before it can strike conducting airway tissue, it became necessary to reach ~200 $\mu g/m^3$ to observe a linear relationship between the respiratory bronchiolar dose and the inhaled tracheal concentration.

By accounting for nasopharyngeal removal, this type of information enables us to put into perspective the exposure concentrations and effects observed in animal studies. It becomes apparent, for example, that a lower tracheal concentration is required to deliver a given dose in man as compared to the rabbit or guinea pig. Thus, our modeling data are useful in planning experiments to fill gaps in the existing data base. (The O_3 criteria document probably reflects the most extensive toxicological data base for any pollutant, yet many gaps remain. Very few studies have looked at a commonality of end points in several species in terms of dose-response curves, etc.)

Many human data have been obtained in studies in which subjects exercised while being exposed to O_3 or nitrogen dioxide (NO_2) (for example: Bates et al. 1972; Hackney et al. 1975). Evaluating these studies with our model shows that, as tidal volume increases, the model predicts a tremendous increase in uptake by respiratory airway tissue. Due to the transit times involved, a relatively constant amount is removed by conducting airway tissue and a decreasing amount is depleted by the mucous. Assuming 750 and 200 $\mu g/m^3$ as the maximum and minimum concentrations, respectively, we can see the vast importance of exercise level on the resulting dose. In many of the human studies, exercise levels that approximately doubled the tidal volume (~1000 ml) were used. Assuming a concentration range of from 200 to 750 $\mu g/m^3$, the model prediction ranges from removal of 45% of the O_3 to delivery of ~80% to respiratory bronchioles (compared to the base line). Such a prediction correlates with increased pulmonary function effects that are indicative of decreasing airways.

Because EPA is charged with protecting individuals at any level of activity and under any exposure conditions, it would be very helpful to know the exposure level required to yield 400 $\mu g/m^3$ at the human trachea. Unfortunately, that information is not currently available. At any rate, if we compare tidal volumes of 500 ml and 1750 ml while the subjects are resting at an 800-$\mu g/m^3$ trachea level and exercising at a 400-$\mu g/m^3$ level, respectively, the model predicts the same maximal dose to the respiratory bronchioles. There are millions of adults jogging and children playing; what are the effects and what are the safe exposure levels in view of such activity levels? There is certainly justification, in the Los Angeles Air Basin, for air pollution alerts and for restrictions on children's play during those episodes. Mathematical modeling of dosage permits us to make meaningful comparisons of experimental results and to better calculate safe exposure levels, thereby helping to determine whether such restrictions are justified for other locations and environmental conditions.

QUANTITATIVE BIOCHEMICAL DATA ON THE MUCOUS LAYER: A MAJOR RESEARCH NEED

Current modeling efforts focus on the sensitivity of dose to mucous production. We are in the process of establishing bounds for NO_2.

The biochemical data required to model a gas greatly influence the amount of useful information that can be obtained. Fortunately, information is available for O_3; O_3 is so reactive that one can consider instantaneous irreversible reactions with many biological substances. Unfortunately, this is not the case for NO_2. Reactions with NO_2 are much slower. With sulfur dioxide (SO_2) there are different reactions to consider, again requiring a lot of information.

To accurately predict how much gas will reach the respiratory bronchial and alveolar regions requires quantitative biochemical data on what is available in the mucous to react with the gas. Such data would enable us to put species differences into perspective in terms of absolute dosage. Most of the available animal data, however, are qualitative in nature. For example, much of the work on mucous secretions has involved staining techniques: we know that something is present but not in what quantity. Some quantitative human data are available (Lewis 1971; Köhler et al. 1969).

The thickness of the mucous layer is one parameter that can impact upon the probability of finding effects. Luchtel (1976) completed a study in rabbits. There is controversy about the thickness of the mucous layer in rats. We also need better data on regional production and transport of mucous.

Effective axial diffusivity relates to the effective diffusion down the airway. Past modeling has incorporated only molecular diffusion; the correct term is in fact ~2000 times greater in the first few generations of the conducting airways. Some work on a human model has been completed by Scherer et al. (1975). For animals, the needed data are (once again) lacking. Also lacking are the diffusion coefficients in mucous and surfactant. Thus far in our modeling, we have been forced to substitute the value for water.

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WORKSHOP COMMENTARY

Question: Do you know the competence of your predictions in terms of error limits? Are the estimates really so precise that you can tell the difference between humans and animals?

F. J. Miller: We plot our results on a log scale. Thus, the increments represent two- and three-fold differences.

Question: You obtained these results from how many studies, for example, on humans?

F. J. Miller: Reaction components are readily available from two papers: Lewis (1971) and Kohler et al. (1969). Again, this is a function of the sensitivity analysis: If the input data are off by a factor of 25, we're in trouble; if they're off by a factor of 10, we're okay. As better data become available, reiteration of the analysis builds confidence in the model's predictions.

Question: Do you intend, in your future work, to give some indication of confidence in the lines you produce? Do you have plots for that?

F. J. Miller: Solving the differential equation does not yield the confidence limits. One can change the variables in the model: frequency, tidal volume, breath-holding, and so on. One can look at all those things. I have just given you a smattering of them.

Question: Do you have an indication, all along the way, of confidence in what you put in?

F. J. Miller: Well, the differential equation applies to gas transport. That's not a unique equation but, rather, the equation that would apply to any coordinate system. We make simplifying assumptions for analyzing the tracheobronchial airways and the rest of the lung. There's nothing "magical" about the first equation.

Comment: Some of the problem here is in going from small animals to large animals. Recognizing that effects in humans are ultimately what we're after, are there (or would you like to see) some large animal studies that would make some of these measurements experimentally and allow you to confirm your models as you go along?

F. J. Miller: We need that kind of information. The limiting factor is the morphometric data. I understand that some data on monkeys are starting to become available. The usefulness of this modeling approach is limited by the availability of morphometric data; beyond that, of course, there is no limit in terms of its application.

J. L. Mauderly: I have three comments. First, with respect to your last statement, I think there are some fairly detailed morphometric data available that range from human down through rodents.

Secondly, I am very glad to see the growing recognition that it's not the air concentration but the inhaled material that causes the effect. We physiologists have been saying this for years. The message is finally coming through that it's not ventilation but alveolar ventilation that's important, and that's a simple relationship that we've been working with for decades.

Finally, Graham [Chapter 28 of this volume] alluded to some studies by age and said that you would be mentioning more about this...

J. A. Graham: The main questions are whether we should use rats or another species, and whether data on rat age susceptibility can be related to humans.

J. L. Mauderly: Yes, that's what I was looking for. My comment is that we are learning more and more with respect to lung structure and function with age in these different experimental animals, but right now we know almost nothing about the senescent lung in most of these species. We do know quite a bit about the dog in terms of morphology, morphometry, and pulmonary function. Very recently, quite a lot of data have been produced on rodents (rats and hamsters; particularly rats). What we see is not very encouraging. We know almost nothing about primates.

Now, certainly we know quite a bit about primate morphology and morphometry and pulmonary function. A group at Davis has done a lot of work in this area, but almost nothing on the aged primate and on the time course of senescence. This is a "glass house" situation because we're doing and planning a lot of studies in rats.

I've said on a number of occasions that the reason for this is that the life span of the average beagle dog is longer than the political life span of the average politician. This is probably about the only reason we're working on rats. Statisticians encourage us to use rats, too.

We know at the present time that the pattern of senescence in lung structure and function in the dog is very similar to that in man, even though the lung type is different. We know just from preliminary data that the pattern is markedly different in rodents (certainly in the rat): there is a growing lung throughout the life span, and this must impact on carcinogenesis and the development of chronic lung disease. And we're skirting around this issue and ignoring it and proceeding as though rats are men--which we know they're not.

We also know, from some recent data, that lung function may peak at mid-age in the rat (~18 mo). Even one group at 27 mo--very close to the expected life span for the Fischer rat--had lung function that was still very good with respect to young adult normals. In contrast, human lung function peaks in young adults and begins a slow decline that is progressive and almost linear throughout the adult life span. That's not the case in rodents; in fact, we don't even know yet if there is a terminal decline in rodent lung structure and function.

This is a comment rather than a question. Actually, it's a sermon. And I think it's something we've got to be very much aware of.

F. J. Miller: I wouldn't deny the need to use other animal species.

J. L. Mauderly: The point is that we don't have this information about the dog (and perhaps the primate). We must continue to fund at least some studies in animals known to have similar patterns of change with age.

C. A. Heckman: Aging studies at Oak Ridge National Laboratory are examining, in a preliminary way, changes in the aging of the rat lung. So far, the computer data indicate that there is probably more cellular septum and greater septum thickness in the rat lung. This may be because the physiological and functional measures are not quite as sensitive in these smaller animals. There may be some more morphological changes in larger animals that can be picked up by functional measurements.

Also, we've found some differences in the way the epithelium from the trachea grows in vitro in aging rats as compared to younger rats. Let me emphasize that this is all preliminary work.

30. OVERVIEW OF CURRENT AND PLANNED RESEARCH BY THE HUMAN STUDIES DIVISION

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INTRODUCTION

The Human Studies Division (Health Effects Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina) is a new entity. A reorganization late in 1978 combined the then existing Population Studies Division, which focused on epidemiologic studies, with the Clinical Studies Division, which was centered mainly in our Human Research Facility in Chapel Hill, North Carolina. These two organizations were combined to create the new Human Studies Division.

The main intent of the reorganization was to enable better coordination and information exchange between our epidemiologic and clinical programs. Both programs are now under a single Director. Hopefully, the net result of the reorganization will be better interaction between these two very different research fields.

EPIDEMIOLOGIC PROGRAM

Previous EPA-supported oxidant studies have focused mainly on the Los Angeles area. Studies have involved the measurement of irritation symptoms in various cohorts, including athletes and schoolchildren.

For the past several years we have funded a study with Copley International to quantify the acute health responses measured by respiratory disease from various patterns of exposure to nitrogen dioxide, ozone, and suspended sulfates and nitrates. The investigators have followed daily reporting of acute respiratory disease symptoms for a period of 26 weeks in 300 families in each of 4 communities. This study should be completed in June 1980.

For the past three years we have funded an effort at Loma Linda University (Loma Linda, California) to replicate a study of cytogenetic effects of photochemical oxidants in college freshmen in the Los Angeles Basin. This study was designed: (1) to determine if there is an increased risk of chromosomal aberration in peripheral lymphocytes among young adults in an area of high ambient photochemical air pollution, (2) to determine if such aberrations persist upon emigration from the polluted area, and (3) to determine if new immigrants develop the chromosomal aberrations. We expect a report on this study in February 1980.

Chapman (Chapter 34 of this volume) describes an ongoing air pollution study in the Texas Gulf Coast area that is designed to assess the effects of air pollution on local asthmatics' symptoms and lung function, and on performance symptoms and physiology in local residents who exercise vigorously and repeatedly in a standardized way.

Dawson (Chapter 39 of this volume) describes an integrated set of human and animal studies planned by the Air Resources Board of the State of California.

CLINICAL PROGRAM

Our clinical research activities with photochemical oxidants are described by Haak, Hazucha, and Ginsberg (Chapters 31, 32, and 33 of this volume). These studies are conducted in our $8-million Human Exposure Facility located in Chapel Hill, North Carolina.

WORKSHOP COMMENTARY

Question: We learned earlier [Chapter 28 of this volume] that certain animal studies will be replicated by EPA. What key human clinical studies mentioned in the O_3 and NO_2 criteria documents will be replicated?

R. S. Chapman: I would guess that the Orehek et al. (1976) study [see Chapter 33 of this volume for details of reference] will be replicated.

R. E. Lee: I think future studies will depend in some measure on the discussions at this workshop. That's one of the reasons we asked you to come.

31. HUMAN PULMONARY ADAPTATION TO OZONE

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INTRODUCTION

Animal and human studies have established that short-term exposure to low levels of ozone (O_3) produces detrimental effects involving multiple organ systems and cell types. In most cases, the health impact of these changes (particularly the long-term consequences) is very unclear. We do not know which of the observed detrimental effects has the greatest impact on health, nor for that matter do we know which of the effects is the most sensitive indicator of detrimental exposure. We do know that the detrimental effects include decreased immuno-integrity of circulating lymphocytes, changes in biochemical parameters, decreased pulmonary mucociliary clearance, and decrements in standard pulmonary function tests.

This report describes a series of studies--the OZADAPT series--that was designed to examine the effects of sequential O_3 exposures on young healthy nonsmoking men. In all, OZADAPT involved five studies and a total of 90 young healthy nonsmoking male subjects. These subjects were examined for pulmonary function measures as well as biochemical and immunologic parameters.

A full presentation of the complex OZADAPT series cannot be given here. This report presents some of the pulmonary function results and compares these findings across the five separate studies, with particular attention to the question of "adaptation" in terms of pulmonary function.

PROTOCOL

Identical screening procedures were used to select the subjects for all five studies. Table 31-1 indicates the mean values and variation of height, weight, and age for each group of subjects. Comparisons of demographic data revealed no significant differences among the groups. Any subject with a history of allergy or asthma was rejected, but no skin tests were performed.

As shown in Table 31-2, each OZADAPT study consisted of five consecutive study days (Days 1 through 5 = Monday through Friday). Day 1 was always a control day; exposures (4 h/d) were performed on Days 2 through 5. OZADAPT I and IV were control studies in which subjects received only air; OZADAPT II, III, and V involved exposures to 0.4 ppm O_3. Pulmonary function data were obtained at three times each day: C_1 (base line), C_2 (2 h), and C_4 (4 h). The pulmonary function data reported in this paper are for forced expiratory volume (FEV_1).

TABLE 31-1. HEIGHT, WEIGHT, AND AGE OF SUBJECTS

Parameter	OZADAPT	Mean	Standard Deviation
height (cm)	I	181.2	6.30
	II	183.6	6.04
	III	181.4	5.84
	IV	180.3	8.99
	V	180.6	7.08
weight (kg)	I	76.49	7.05
	II	76.55	9.72
	III	77.55	11.41
	IV	71.61	9.64
	V	72.79	8.88
age (yr)	I	25.24	2.58
	II	24.83	2.34
	III	25.89	1.96
	IV	25.03	2.83
	V	24.16	2.63

RESULTS AND DISCUSSION

We observed significant differences in mean pulmonary function for OZADAPT II (light exercise) versus OZADAPT III (heavy exercise). The mean FEV_1 data from OZADAPT II indicated a much lower decrement at the light (35 liters/min) exercise level. The mean response diminished to an insignificant level by Day 5, the fourth consecutive O_3 exposure day. In contrast, the mean FEV_1 data from OZADAPT III showed a much larger decrement at the heavy exercise (57 liters/min) level; subjects did not return to base-line values by 24 h after Day 2, the first O_3 exposure day. The response to the second exposure (Day 3) was similar in magnitude of mean decrease. By the fourth day

TABLE 31-2. EXPERIMENTAL PROTOCOL

OZADAPT	Day	Exposure[a]	Treadmill Exercise[b]
I	1	Air	light
	2	Air	light
	3	Air	light
	4	Air	light
	5	Air	light
II	1	Air	light
	2	O_3	light
	3	O_3	light
	4	O_3	light
	5	O_3	light
III	1	Air	heavy
	2	O_3	heavy
	3	O_3	heavy
	4	O_3	heavy
	5	O_3	heavy
IV	1	Air	heavy
	2	Air	heavy
	3	Air	heavy
	4	Air	heavy
	5	Air	heavy
V	1	Air	heavy
	2	O_3	none
	3	O_3	none
	4	O_3	heavy
	5	O_3	heavy

[a] 4 h/d. O_3 concentration = 0.4 ppm.

[b] Light treadmill exercise = 4 mi/h, 0% grade, 15 min, 35 liters/min ventilation. Heavy treadmill exercise = 4 mi/h, 10% grade, 15 min, 57 liters/min ventilation.

of consecutive O_3 exposure (Day 5), however, the mean decrement had diminished to little more than the variability in the control values.

Therefore, on the basis of mean decrement in FEV_1 across groups, one may conclude that consecutive exposures are associated with a progressive diminution in observed decrement. However, a consideration of individual subject values across the week points up the great importance of individual sensitivities, even among "normal" vigorous young men.

Figure 31-1 displays three individual patterns that were observed. (All subjects received identical treatment, exercise, etc.) Subject #3 was barely responsive across all exposure days. Subject #7, on the other hand, was very responsive on the first and second exposure days, but his reactivity was greatly diminished by the third and fourth exposure days. Subject #8 was equally reactive on the first and second exposure days; more importantly, his responsiveness persisted. This clinical observation of wide variance among "normal" young men prompted us to undertake the additional study (OZADAPT V), which was designed to further characterize the responsiveness of these subjects.

In OZADAPT V, mean FEV_1 responses were minimal for Days 2 and 3 (rest). There was a large response on Days 4 and 5 (heavy exercise). Decrements on Days 4 and 5 were very similar to those observed on Days 2 and 3 in OZADAPT III. Unfortunately, we could not continue the OZADAPT V exposures for a fifth and sixth day to look for "adaptation."

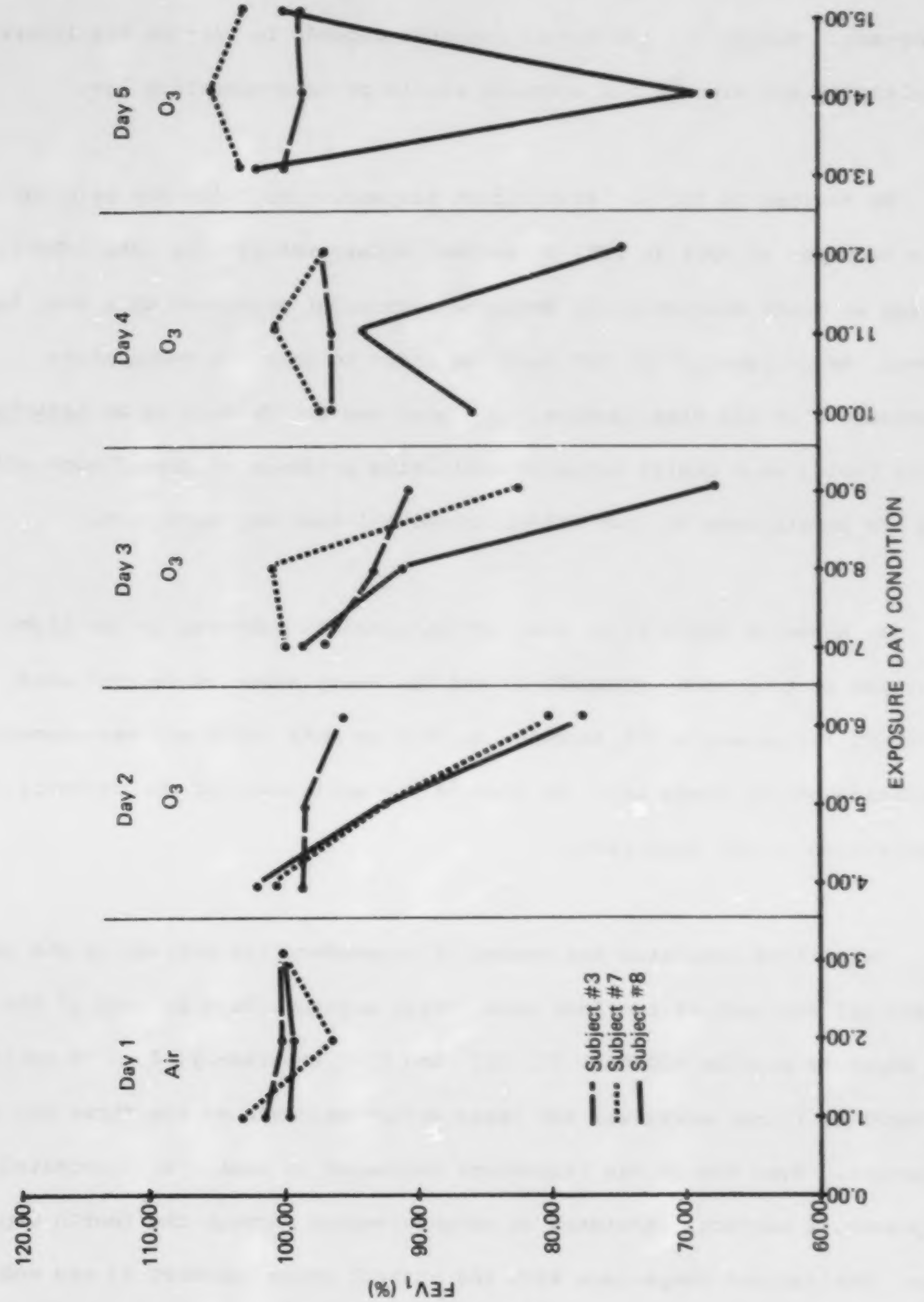

Figure 31-1. Response variability among three subjects in OZADAPT III.

The results of OZADAPT V were not unexpected, but they are extremely important. Whether an individual responds depends in part on his inherent sensitivity and also on his exercise challenge or provocation level.

We decided to define "significant responsiveness" (in the clinical sense) as a decrease of >20% in FEV_1 or maximal midexpiratory flow rate (MMEF), even though we could statistically define a meaningful decrement at a much lower level. We designated the 20% level in order to permit a comparative examination of all five studies. Our goal was to identify on an individual basis (not a mean basis) subjects exhibiting evidence of significant effect and the persistence of that effect across all four exposure days.

As shown in Table 31-3, none of the total 45 subjects in the light exercise control study (OZADAPT I) and the heavy exercise control study (OZADAPT IV) showed a 20% decrease in FEV_1 or MMEF under any measurement condition on any study Day. In view of the strictness of our criteria, this observation is not surprising.

Table 31-4 indicates the number of responders (as defined by the same criteria) for each of the four consecutive exposure days in each of the three O_3 exposure studies (OZADAPT II, III, and V). Remarkably, 4 of 15 subjects in OZADAPT II (light exercise) met these strict criteria on the first day of exposure. Even though the responders decreased in number with repeated exposure, 2 subjects persisted in responsiveness through the fourth exposure day. Statistical comparison with the control group (OZADAPT I) was not

TABLE 31-3. SIGNIFICANT RESPONSIVENESS[a] AMONG CONTROL SUBJECTS

OZADAPT	Day	Number of Significant Responders
I	2	0
	3	0
	4	0
	5	0
IV	2	0
	3	0
	4	0
	5	0

[a] Defined as a decrease of >20% in FEV_1 or MMEF.

significant because of the small number of subjects. In OZADAPT III, 7 out of 15 subjects were initial responders. Once again we observed a decrease in number of responders with continued exposure, although 2 subjects were responsive even after the fourth exposure day. In OZADAPT V, there were very few responders on the first and second exposure days (rest). On the third and fourth exposure days (heavy exercise), the numbers of responders were very similar to those in the first and second exposure days of OZADAPT III. From this, it is quite apparent that the ventilation level of exercise challenge is an important provocative stress that may evoke individual sensitivity to O_3 exposure. Note also that, even with this challenge, one third of the OZADAPT V subjects did not respond in terms of our strict criteria.

TABLE 31-4. SIGNIFICANT RESPONSIVENESS[a] AMONG SUBJECTS EXPOSED TO OZONE

OZADAPT	Day	Number of Significant Responders	Probability
			(I vs. II:)
II	2	4/15	0.010
	3	2/15	0.111
	4	1/15	0.341
	5	2/15	0.111
	total	4/15 (27%)	0.010
			(III vs. IV:)
III	2	7/15	0.003
	3	7/15	0.003
	4	4/15	0.050
	5	2/15	0.241
	total	8/15 (53%)	0.001
			(V vs. IV:)
V	2	1/15	0.500
	3	3/15	0.112
	4	9/15	<0.001
	5	7/15	0.003
	total	10/15 (66%)	<0.001

[a] Defined as a decrease of >20% in FEV_1 or MMEF.

CONCLUDING REMARKS

Investigators are only beginning to understand the many factors which affect pulmonary function during short-term acute exposures to O_3 and other oxidants. Among these factors are: concentration of the gas, ventilation (exercise) level, duration of exposure, proximity of pulmonary function testing to exposure peaks, attitude, previous exposure experience, humidity, temperature, and many others.

Our OZADAPT studies stress that individual sensitivity is also very important. The fact that the OZADAPT subjects were not "high-risk" subjects underlines the need to investigate the presumably large population subsets that are apparently intolerant of O_3 exposure.

WORKSHOP COMMENTARY

R. S. Chapman: Has there been any subsequent follow-up on OZADAPT subjects who seemed not to "adapt" like the group means, with respect to possible atopic tendency not uncovered in the initial examination?

E. D. Haak: Are you asking how many of our responsive people were discovered (say, by skin testing) to be truly allergic, and how many of the nonresponsive were found to be not allergic?

R. S. Chapman: That would be a subquestion. Having discovered that some of the subjects were indeed not "adapting," did you do any follow-up skin or other testing to reveal what we might call an "occult allergic tendency"?

E. D. Haak: We have not gone back and done any skin testing on those individuals who appeared to be persisting in their responses to O_3 exposure. As a matter of fact, we haven't gone back and done any follow-up testing of any kind on any OZADAPT subjects. Rather, we have been involved in an analytical appraisal of the study. We might be able to go back and do some follow-up testing. Unfortunately, our subject population is a transient student population; many of them have graduated and would not be available for testing.

32. OZONE-INDUCED HYPERREACTIVITY AS MEASURED BY HISTAMINE CHALLENGE IN NORMAL HEALTHY SUBJECTS

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INTRODUCTION

Haak (Chapter 31 of this volume) has demonstrated that 0.4 ppm ozone (O_3) significantly decreases pulmonary performance in human subjects who are exercising. Even under resting conditions, 0.6 ppm O_3 for 1 or 2 h results in a significant impairment in group mean pulmonary function. However, examination of each individual's performance under such conditions reveals considerable variation in the degree of response to O_3 exposure.

In previous studies, we observed that some subjects show little change even at O_3 concentrations as high as 0.8 ppm, while other, apparently more sensitive subjects show decreases of up to 50-60% in maximal midexpiratory flow rate following 2 h of exposure with exercise (Bates and Hazucha 1973). Clearly, such a wide spectrum of individual variability is obscured if effects are evaluated only as group mean responses. We also noted that the duration of effect is only a matter of hours: we were not able to detect by spirometric means any residual effects at 24 h post-exposure (Bates and Hazucha 1973). Recently, however, Golden et al. (1978) demonstrated that O_3 exposure can lead to airway hyperreactivity that persists for several days (and sometimes weeks) even in subjects who exhibit no continuing effects on pulmonary mechanics.

Consequently, the study reported here attempted to answer two basic questions: (1) Does O_3 exposure alter airway reactivity as assessed by a standard bronchial challenge technique using histamine aerosol (Chai et al. 1975)? (2) Is there any correlation between individual sensitivity to O_3 and degree of airway reactivity?

PROTOCOL

The subjects were 14 healthy individuals having no history of allergies or asthma. A 3-d protocol was employed. On Day 1, subjects received a 2-h air (control) exposure. On Day 2, subjects received a 2-h exposure to 0.6 ppm O_3. Each exposure period included two 15-min periods of treadmill exercise (4 mi/h x 10% grade x 15 min = 1 mi walk). On Day 3, the subjects returned for 24-h follow-up testing.

A battery of pulmonary function tests (PFT's) was performed prior to each exposure, 2 h post-exposure, and 24 h post-exposure. The PFT's included: functional residual capacity (FRC) and airway resistance (RAW) acquired in a plethysmograph, several measures of forced vital capacity (FVC), vital capacity (VC), forced inspiratory volume (FIV), and maximal voluntary ventilation (MVV) measured by a dry seal spirometer. During exposure to air or O_3, FVC and RAW were measured at 1 and 2 h of exposure, 10 min after the preceding exercise period. In the post-exposure 2-h recovery period, FVC was monitored at 30-min intervals.

The PFT battery was always followed by a complete histamine challenge test. Histamine aerosol was generated in a De Vilbiss #65 ultrasonic nebulizer. The aerosol was passed through an impactor chamber in order to narrow the particle size distribution to a mass median aerometric diameter of 2.0 ± 1.6 µm. Seven different concentrations of histamine were generated: 0.0 (saline), 0.3, 0.6, 1.25, 2.5, 5.0, and 10.0 mg histamine salt/ml aerosol. Briefly, the challenge test consisted of inhaling 5 normal tidal breaths (800-1000 ml/breath) of each concentration at 5-min intervals. One FVC and one RAW measure were obtained 2 min after inhalation of each concentration.

RESULTS

Figure 32-1 shows the mean response and recovery of 14 subjects to O_3 as determined by changes in forced expiratory volume (FEV_1). The broken line shows the mean control (air exposure) data (± 1 S.D.) while the solid line represents the mean O_3 exposure data. Controls showed no statistically significant changes over the exposure and recovery periods; the maximum absolute difference between mean FEV_1 values was only 270 ml. In contrast, O_3

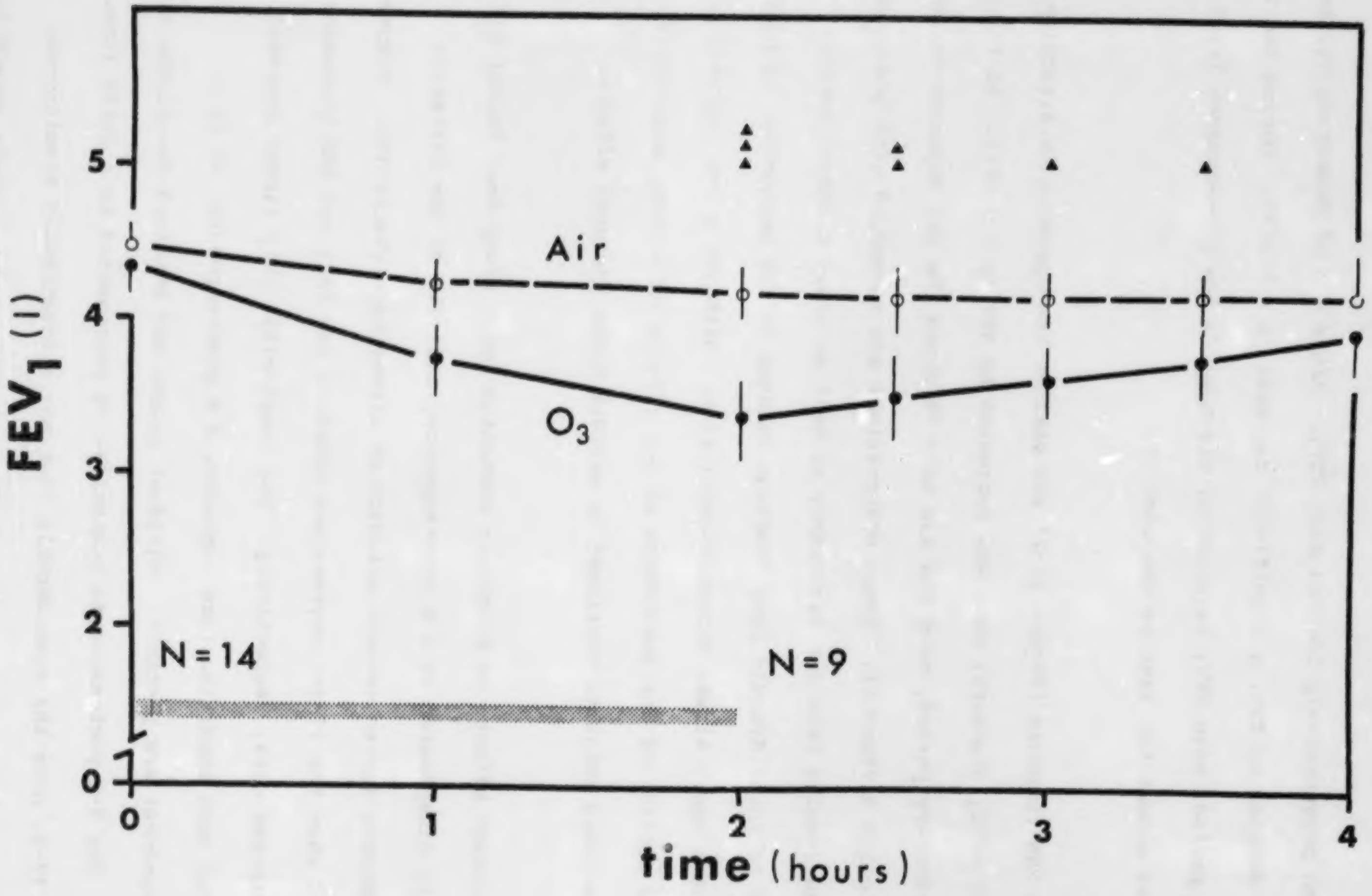

Figure 32-1. Mean FEV$_1$ in 14 human subjects during and after exposure to air and O$_3$. Solid line = 2-h exposure to 0.6 ppm O$_3$; broken line = 2-h exposure to air (control).

inhalation progressively lowered mean FEV_1. After 2 h of breathing O_3, mean FEV_1 had dropped by 23%, a significant decrease ($p < 0.001$). During the 2-h recovery period, mean FEV_1 returned slowly towards the pre-exposure level and at 2 h was almost the same as the control.

The RAW response (Figure 32-2) was similar but opposite in direction. After 2 h of O_3 exposure, mean RAW increased by 45% ($p < 0.001$). At the end of the recovery period, mean RAW was only 7% above the pre-exposure value (not statistically different). These observations are in accord with previously published results from our laboratory as well as other research centers. Implicit in these dynamic lung function changes is the production by O_3 of significant upper airway bronchoconstriction. Although a 2-h recovery period appeared sufficient for subsidence of the effects on a group mean basis, some of the exposed subjects continued to exhibit minor residual effects.

Although effects on pulmonary mechanics (on a group mean basis) had virtually disappeared at 2 h post-exposure, analysis of the histamine dose-response curves reveals evidence of airway hyperreactivity. Figures 32-3 and 32-4 show the linear regressions obtained for FEV_1 and RAW histamine dose-response data, respectively. For simplicity, only linear regression lines for each condition (pre-exposure, 2 h post-exposure, or 24 h post-exposure) are plotted; individual points and standard deviations are not shown. The 2-h post-exposure regression is represented as a solid line. In Figure 32-3, note the considerable (but not statistically significant) differences in both intercept and slope of FEV_1 at 2 h post-O_3 versus the

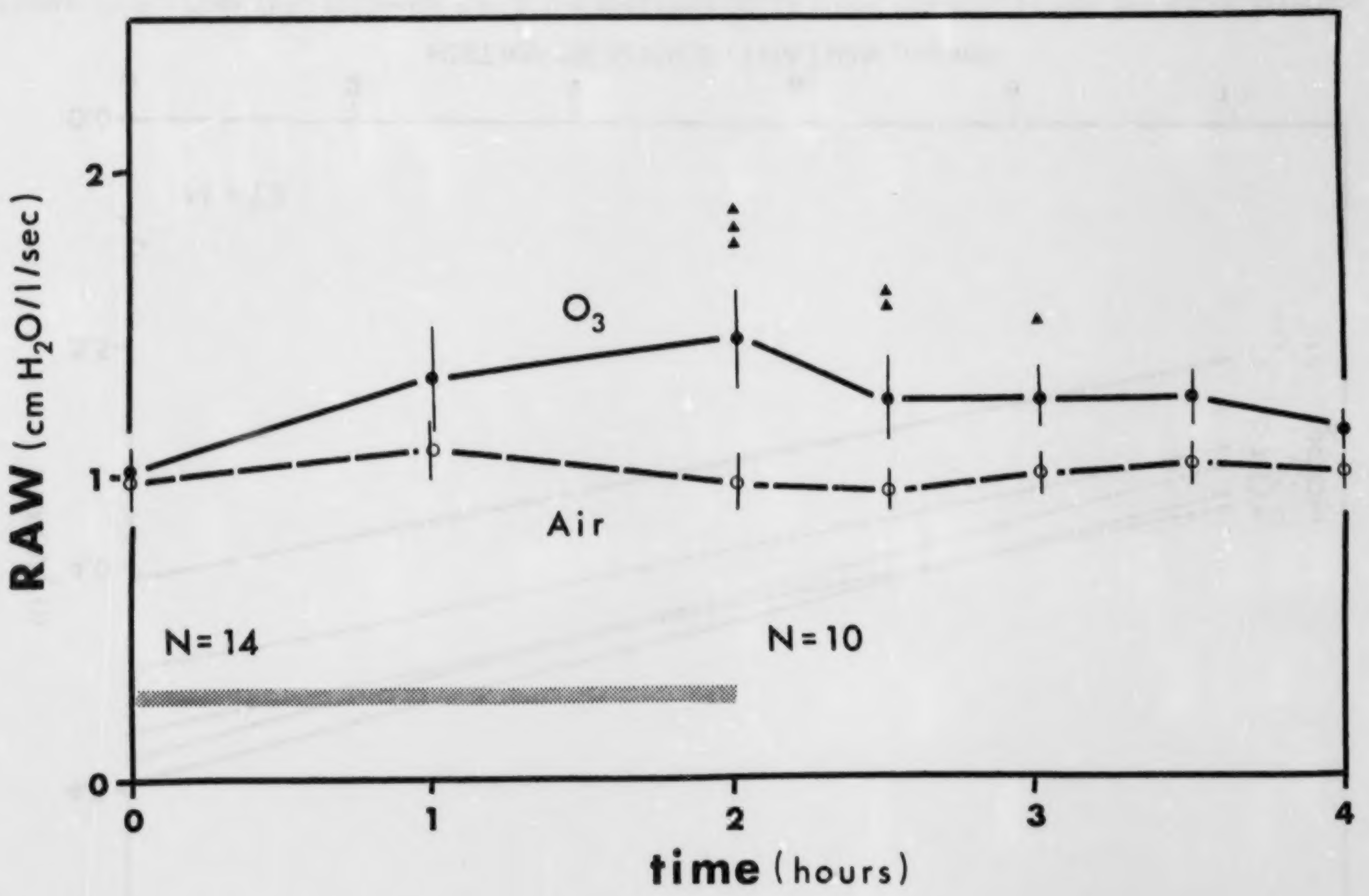

Figure 32-2. Mean RAW in 14 human subjects during and after exposure to air and O_3. Solid line = 2-h exposure to 0.6 ppm O_3; broken line = 2-h exposure to air (control).

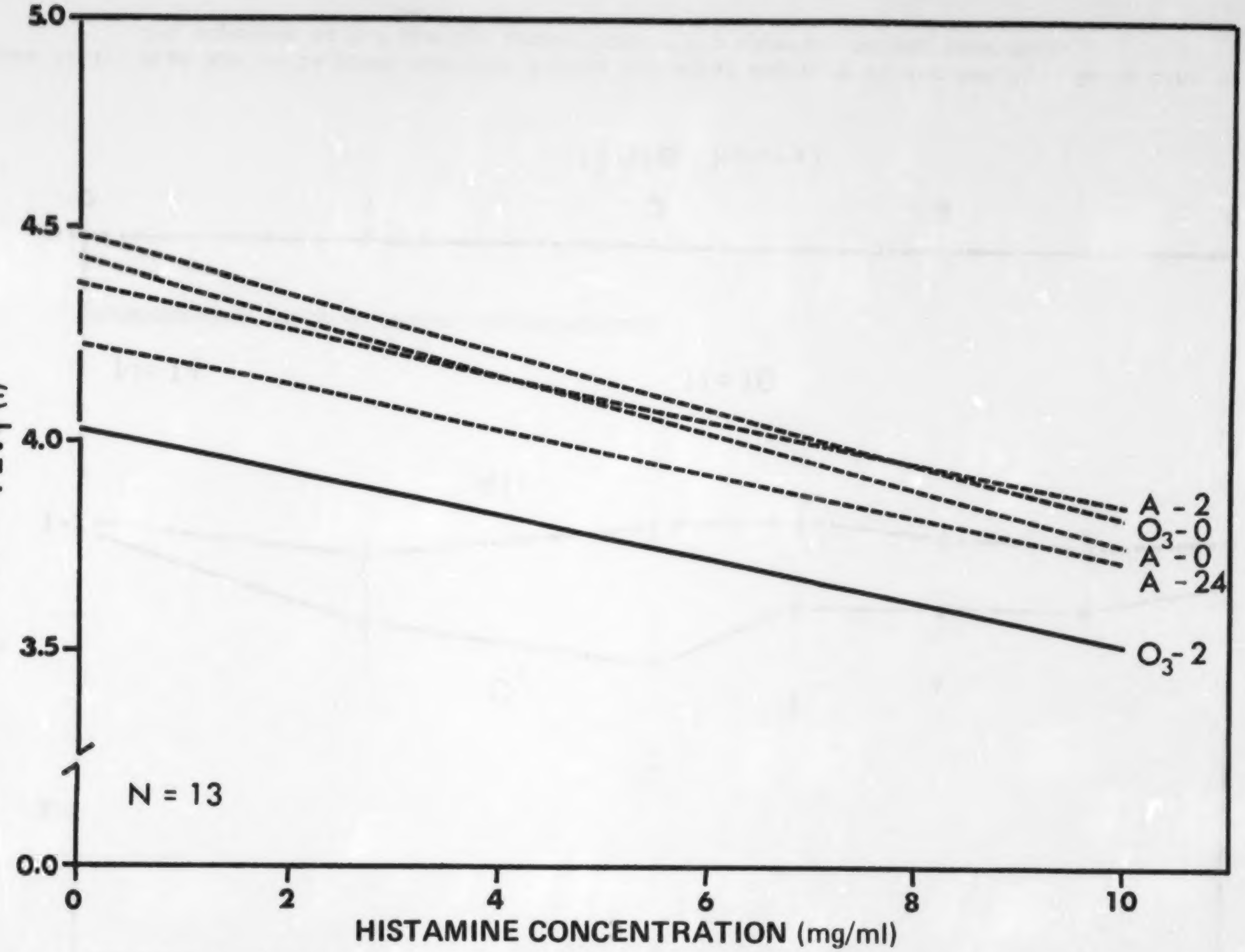

Figure 32-3. Mean FEV$_1$ following histamine challenge in 13 human subjects before and after exposure to air and O_3. A = 2-h exposure to air (control); O_3 = 2-h exposure to 0.6 ppm O_3. 0 = pre-exposure; 2 = 2 h post-exposure; 24 = 24 h post-exposure.

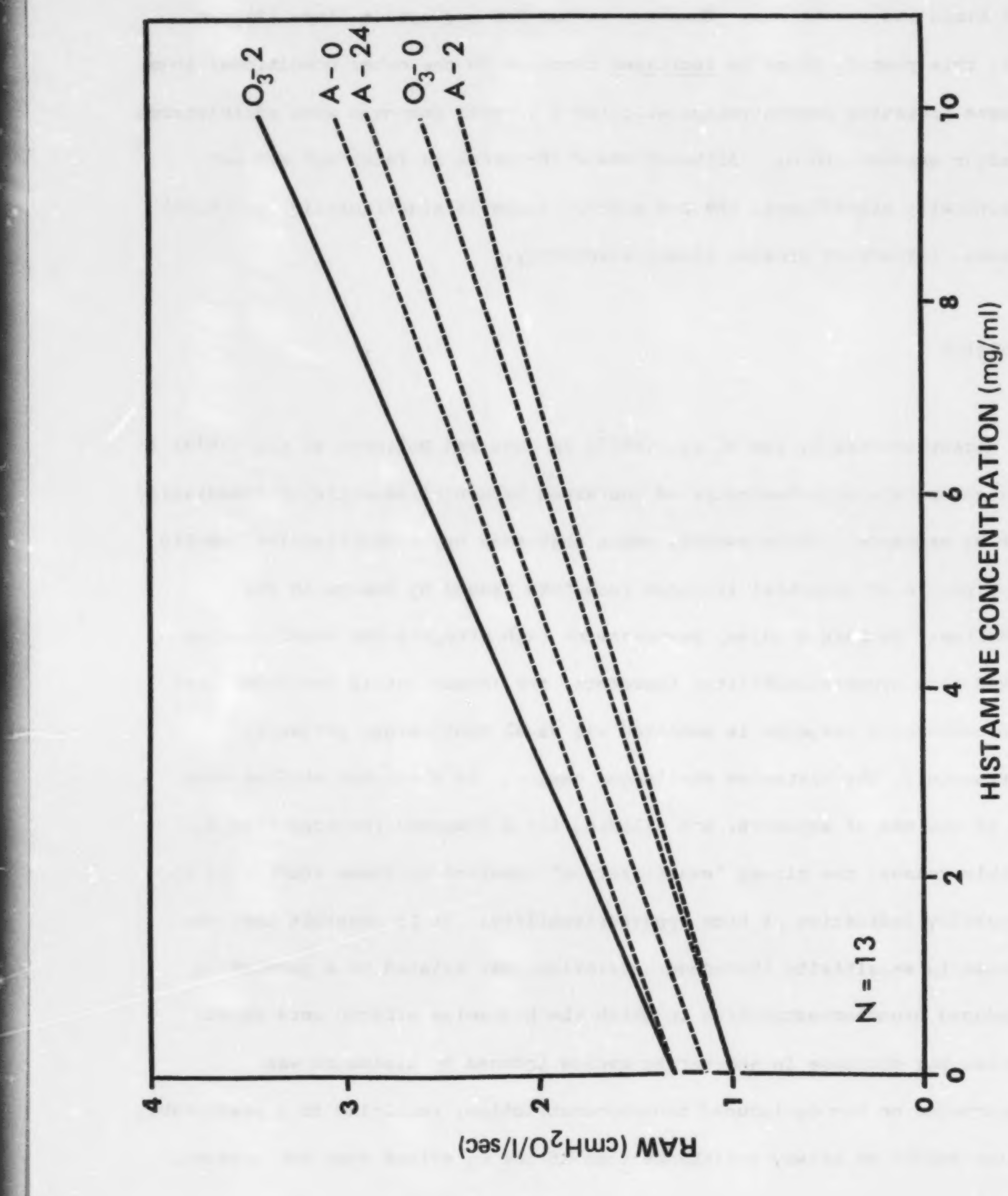

Figure 32-4. Mean RAW following histamine challenge in 13 human subjects before and after exposure to air and O_3. A = 2-h exposure to air (control); O_3 = 2-h exposure to 0.6 ppm O_3. 0 = pre-exposure; 2 = 2 h post-exposure; 24 = 24 h post-exposure.

other times and conditions. However, in the RAW regression lines (Figure 32-4), this post-O_3 slope is increased compared to the other conditions; thus, the same histamine concentration elicited a greater response when administered 2 h after exposure to O_3. Although the differences in intercept are not statistically significant, the 2-h post-O_3 slope is significantly ($p < 0.05$) elevated, reflecting greater airway reactivity.

DISCUSSION

Recent studies by Lee et al. (1977) in dogs and Holtzman et al. (1979) in human nonsmokers have demonstrated increased bronchial sensitivity immediately after O_3 exposure. These authors state that such hypersensitization results from exposure of bronchial irritant receptors caused by damage to the epithelium. In both studies, pretreatment with atropine and vagal cooling blocked this hyperirritability; therefore, the investigators concluded that the bronchomotor response is mediated via vagal cholinergic pathways. Unfortunately, the histamine challenges employed in these two studies were done at the end of exposure, not allowing for a complete recovery from O_3. For this reason, the airway "sensitization" observed in these studies is not necessarily indicative of true hyperirritability. It is possible that the increase in sensitivity (bronchoconstriction) was related to a persisting O_3-induced bronchoconstriction to which the histamine effects were added. That is, the decrease in the airway radius induced by histamine was superimposed on the O_3-induced bronchoconstriction, resulting in a predictably greater effect on airway resistance than if the O_3 effect were not present.

However, Habib et al. (1979) explored the variability of airway response to histamine and concluded that base-line bronchomotor tone is not a major contribution to the increase of resistance. In our own studies, we allowed pulmonary function to return to the pre-exposure level, in order to avoid this confounding of measurements with double intervention (O_3 and histamine). Even though FVC, FEV_1, and RAW values reached control levels, however, the airways remained hypersensitive. Such increased sensitivity may last up to several weeks in some subjects (Golden et al. 1978).

Closer examination of individual responses in our study showed a wide spectrum of sensitivity following O_3. Some subjects did not appear to respond either to histamine or to O_3, while a few subjects reacted strongly to both challenges. On reviewing the individual responses to both O_3 and histamine (considered separately), the subjects appear to fall into two distinct categories based on type of response: reactors (decrease in FEV_1 of >20% and increase in RAW of >100% compared to control values) and nonreactors (the remaining subjects). When the same separation criteria are applied to the histamine dose-response data, subjects who responded to O_3 seem to have been more reactive to histamine. This reactivity was further enchanced by O_3 exposure. Despite incomplete data analysis, the correlation between response to O_3 and enhanced response to histamine is striking.

Several plausible explanations for this apparent interdependence of histamine reactivity and O_3 response have been explored by other investigators (Golden et al. 1978). It is possible that O_3-induced peripheral airway obstruction causes maldistribution of the aerosol, with a greater proportion going to the larger airways and consequently eliciting increased response. Ozone damage of epithelial cells with consequent exposure of vagal sensory receptors is another possible mechanism (Holtzman et al. 1979). Such denuded receptors may be more sensitive to inhaled histamine. Accordingly, some investigators (Orehek et al. 1977) have proposed the analysis of data in terms of sensitivity and reactivity to histamine. Our data have not yet been analyzed in this manner; therefore, it is difficult to make an in-depth evaluation of various mechanisms contributing to the magnification of response.

Regardless of mechanism, these studies demonstrate that 0.6 ppm O_3 will not only impair dynamic lung function but also increase at least transiently the sensitivity of the tracheobronchial tree. Such hypersensitivity may last well beyond restoration of lung function as determined by spirometry. The high correlation between O_3 reactivity and magnitude of histamine response leads us to conclude that the histamine bronchial challenge can be used as a screening test for O_3 reactivity, and might also predict reactivity to other oxidants.

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WORKSHOP COMMENTARY

J. D. Hackney: Were the subjects exercising or resting?

M. J. Hazucha: The subjects were exercising twice: during the last 15 min of the first hour and during the last 15 min of the second hour of exposure.

J. D. Hackney: To follow up on your dose-response findings, did you test the subjects' responsiveness to histamine 24 or 48 h later?

M. J. Hazucha: Yes, we tested responsiveness at 24 h post-exposure. There still appeared to be increased responsiveness to histamine, but the correlation between O_3 response and response to histamine was smaller.

J. D. Hackney: And at 48 h?

M. J. Hazucha: We didn't do any testing at 48 h.

R. K. Wolff: Did you make any measurement of the actual inhaled dose of histamine as opposed to the concentration in the nebulizer?

M. J. Hazucha: Although we know how much of the aerosol was inhaled, we really don't know how much of it was retained. We believe that if the subject inhaled approximately the same amount of aerosol at each dose, most likely the same amount of aerosol was retained and thus the doses were comparable. I wish we could do such measurements. It is possible, but we lack the technical means at present.

R. K. Wolff: Did you compare the histamine and the methacholine bronchial challenges?

M. J. Hazucha: We did. In another study (using exactly the same protocol) we challenged subjects with methacholine. The magnitude of response seemed to be about the same as with histamine, and we were again able to identify responders and nonresponders. The data are not yet completely analyzed so I cannot give you exact figures.

Question: Do you plan to use 0.25 ppm O_3 in your system?

M. J. Hazucha: Yes, in our forthcoming O_3 threshold study.

Comment: Are you referring to O_3-plus-NO_2 studies? We have plans to look at a study similar to the Orehek study, to try to confirm or deny those observations. We will also look at 0.1 ppm O_3 to confirm or deny the report of De Lucia and Adams. This should follow on the heels of the study discussed by Ginsberg [Chapter 33 of this volume]. We expect to start with a low-level O_3 exposure which should feature the same points made by Haak [Chapter 31 of this volume].

S. V. Dawson: Did you see an apparent increase in the variance of the results under O_3 exposure? This is something that I see recurring fairly often. It's not the mean but actually the variance that's changing, suggesting possibly that some kind of a control system is becoming unstable. A random effects model--a standard statistical model which isn't used much in biology--examines whether variance is significantly changed from one regime to another. Have you looked at that in your O_3 studies?

M. J. Hazucha: No, we have not yet statistically analyzed these variances. We are indeed planning to do in-depth statistical analyses of the variances of single and group data. Specifically, we will look at correlations between responders and nonresponders; we will evaluate the two group responses within and between days.

The increased variance which appeared during exposure and diminished during recovery was caused, we believe, by the spread of responses induced by O_3. Some of the subjects reacted considerably, while some didn't respond at all. Your suggestion that we analyze these variances is very well taken.

M. Goldman: In relation to Dr. Dawson's question on variability, [Figures 32-1 and 32-2] indicate the number of subjects to be ~14 in the beginning but only ~9 during recovery. This may be an explanation for the variability change. Were the same number of individuals measured throughout exposure and recovery?

M. J. Hazucha: Yes. Fourteen subjects were studied from the very beginning to the very end. In some conditions, however, we were unable for technical reasons to recover data. That's why [the figures] show only 9 of the 14 subjects included in the recovery period.

33. RESPONSE OF NORMALS AND ASTHMATICS TO LOW-LEVEL NITROGEN DIOXIDE

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INTRODUCTION

The oxides of nitrogen are important constituents of air pollution; nitrogen dioxide (NO_2) is particularly important. Our interest in defining the adverse effects of low-level NO_2 exposure was stimulated by the increased use of diesel fuel in transportation and power plants.

High levels of NO_2 are known to cause inflammatory lung disease in humans and are implicated as carcinogens in animals. Lower levels, such as those in the ambient air, obviously need further evaluation in man, particularly in sensitive individuals (i.e., those most likely to be at risk). A recent French study (Orehek et al. 1976) suggested that 0.1 ppm NO_2 may be harmful to asthmatics. This finding is at variance with previous work showing no harmful effects at levels of up to 1 ppm in normal human subjects. In the Orehek study, 20 mild to moderately severe asthmatics, aged 17 to 44, were exposed for 1 h to 0.1 ppm NO_2 and air at weekly intervals. The protocol also included nonspecific bronchial challenge with carbacholine, a synthetic analog of the naturally occurring neurotransmitter acetylcholine. Two bronchial challenges were performed in each subject. In terms of specific airway resistance (SRAW), NO_2 exposure caused the dose-response curve to shift to the left, and lower threshold doses of carbacholine were required to cause increases in SRAW. From this it was concluded that very low levels of NO_2 can adversely affect some asthmatics. This conclusion suggests that stringent regulatory standards, particularly a 1-h standard, deserve consideration.

PROTOCOL

In an effort to corroborate these findings, we designed a similar study that is now in progress. Eventually, 15 normals and 15 asthmatics will have been exposed to 0.1 ppm NO_2 and to air (each for 1 h) in a randomized, double-blind, crossover design.

Our asthmatics range from asymptomatic to mildly severe. Where they are asymptomatic, the diagnosis is based on a history of reversible wheezing or dyspnea that is clearly seasonal and not associated with upper respiratory tract infection. All of our asthmatic subjects have at least one positive cutireaction to a battery of allergens common to our area. Our normals have no symptoms or family history of atopy, and all have negative skin tests to the same battery of allergens. All subjects are nonsmokers.

Subjects abstain from all medications for at least 48 h, and all theophylline-containing foods are withheld for 20 h. Asthmatics are not studied within 4 weeks of bronchodilator use, and none are taking steroids. No subject is studied within 4 weeks of symptoms of an upper respiratory tract infection.

Bronchial challenges are performed as recommended by Chai et al. (1975) modified for an ultrasonic aerosol generator (De Vilbiss #65). We use twofold-increasing concentrations of acetyl methacholine (hereafter referred to as "methacholine") for seven doses. The normals receive a first dose of 0.31 mg/ml and a maximum dose of 20 mg/ml. The asthmatics receive a first dose of 0.07 mg/ml and a maximum dose of 5 mg/ml. Five tidal-sized inhalations with a 4-s breath hold are used to ensure a high percentage of particle retention. Within 5 to 6 min of each dose, airway resistance (RAW) and thoracic gas volume (TGV) are measured plethysmographically by the technique of DuBois et al. (1956).

Our major departures, then, from the Orehek study are: (1) inclusion of normals; (2) skin testing to exclude occult atopy in the normal group and to ensure that all the asthmatics share a reagenic mechanism; (3) less clinically severe asthma in the asthmatic subjects; (4) bronchial challenge with methacholine (which has a shorter duration of activity due to its affinity for the enzyme acetylcholinesterase) instead of carbacholine; and (5) more bronchial challenges (including pre-exposure and 24 h post-exposure) at varying concentrations.

Bronchial challenge produces dose-response curves which can be analyzed for the threshold dose of methacholine (defined as the dose causing a 100% increase in SRAW) and for the slope of the line connecting responses at doses above the threshold. While the mechanisms are not clear, changes in these parameters are gaining favor with investigators as a reflection of the adverse effects of oxidant pollutants. Of course, we must remember that methacholine-induced bronchospasm does not reproduce an asthmatic attack (which is certainly more complex than simple smooth muscle contraction). However, methacholine-induced bronchospasm may simulate asthma closely for a very short period of time (e.g., 15-30 min). It is also important to remember that a methacholine-induced increase in SRAW does not necessarily relate to increased morbidity among the asthmatic population.

PRELIMINARY RESULTS

We have obtained and plotted some preliminary data for four asthmatics and four normal subjects. In Figures 33-1 through 33-5, specific airway resistance (RAW x TGV/1000) (SRAW) is plotted linearly on the ordinate, and the methacholine dose is plotted logarithmically on the abcissa. The logarithmic dose plot allows us to stretch the early part of the curve that would otherwise (for asthmatic subjects) go up very rapidly and be compressed at the left-hand side of the graph. The term "PDAR" appears in some figures and is used to describe bronchial challenge. The PDAR base line represents the first challenge given to each subject. The PDAR 4 line indicates another bronchial challenge performed in every subject at least 5 d after the base-line bronchial challenge. (A minimum of 5 d was chosen because of compatibility with previous work and good reproducibility of bronchial challenge at 7-d intervals.) The PDAR 5 line represents a challenge performed 24 h after PDAR 4; PDAR 6 is a challenge performed 24 h after PDAR 5. Exposure to either air or NO_2 occurred immediately before the second of these three PDAR's. To summarize, the PDAR base line represents a dose response at some time distant from the other three, which are dose responses taken at 24-h intervals with an exposure to either air or NO_2 occurring just before the second of the three.

Figure 33-1 represents an asthmatic subject exposed to air and challenged with 2.5 and 5 mg/ml methacholine. Note that the base line appears shifted to the right compared to the pre-exposure PDAR 4 curve. The PDAR 5 curve is similar in appearance. Arrows mark each threshold dose. The threshold doses and the slopes of the lines beyond the thresholds are similar for PDAR 4 and 5. The PDAR 6 curve, however, has a somewhat different appearance, with a higher threshold and a reduced slope--in short, a shift to the right. This trend toward a depression of reactivity on the third of three consecutive days seems to be real and may reflect a repeat treatment effect of the methacholine challenge. To assume that bronchial responsiveness is similar from week to week, then, may not be valid.

Figure 33-2 (two normal subjects) displays only the dose responses obtained immediately after air and NO_2, 1 week apart (i.e., two of the total seven bronchial challenges done in each subject). Note that both subjects

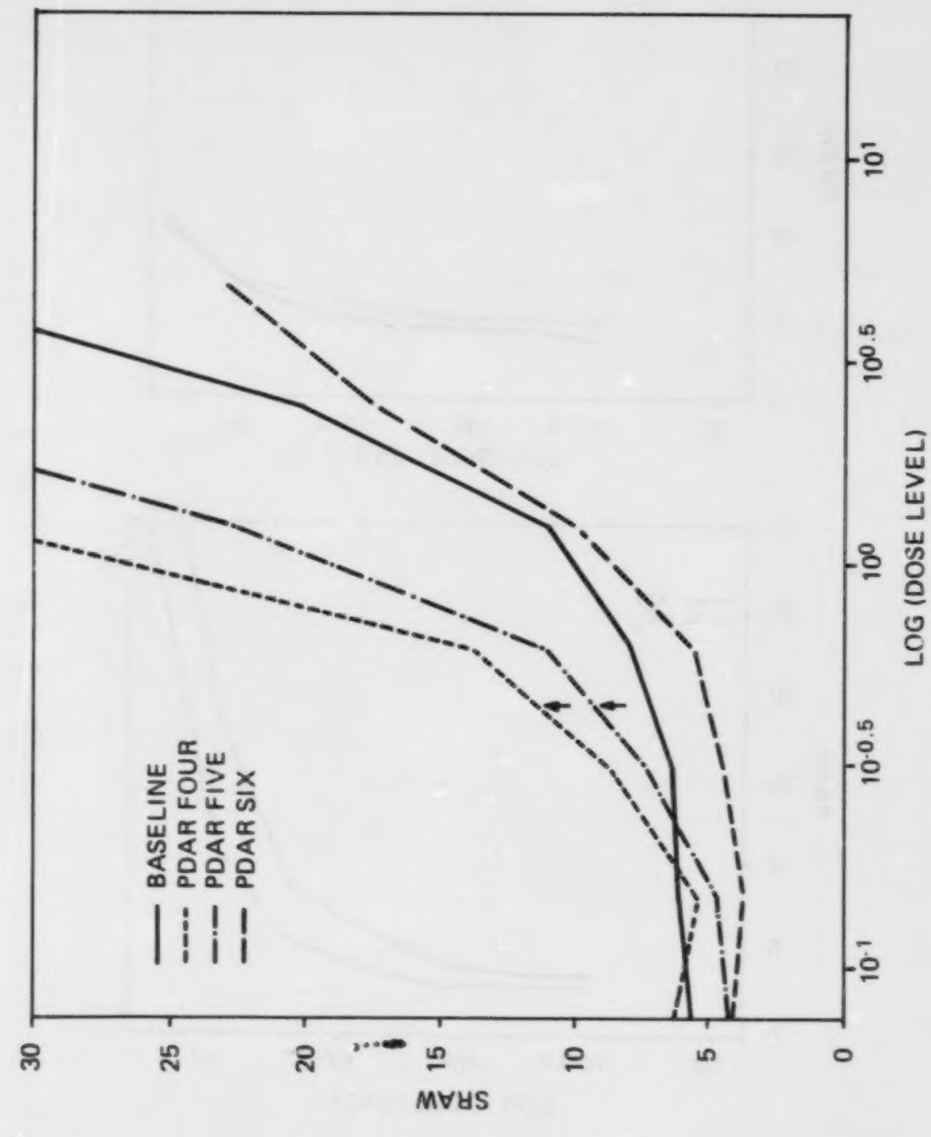

Figure 33-1. Specific airway resistance following three bronchial challenges in an asthmatic subject exposed to air.

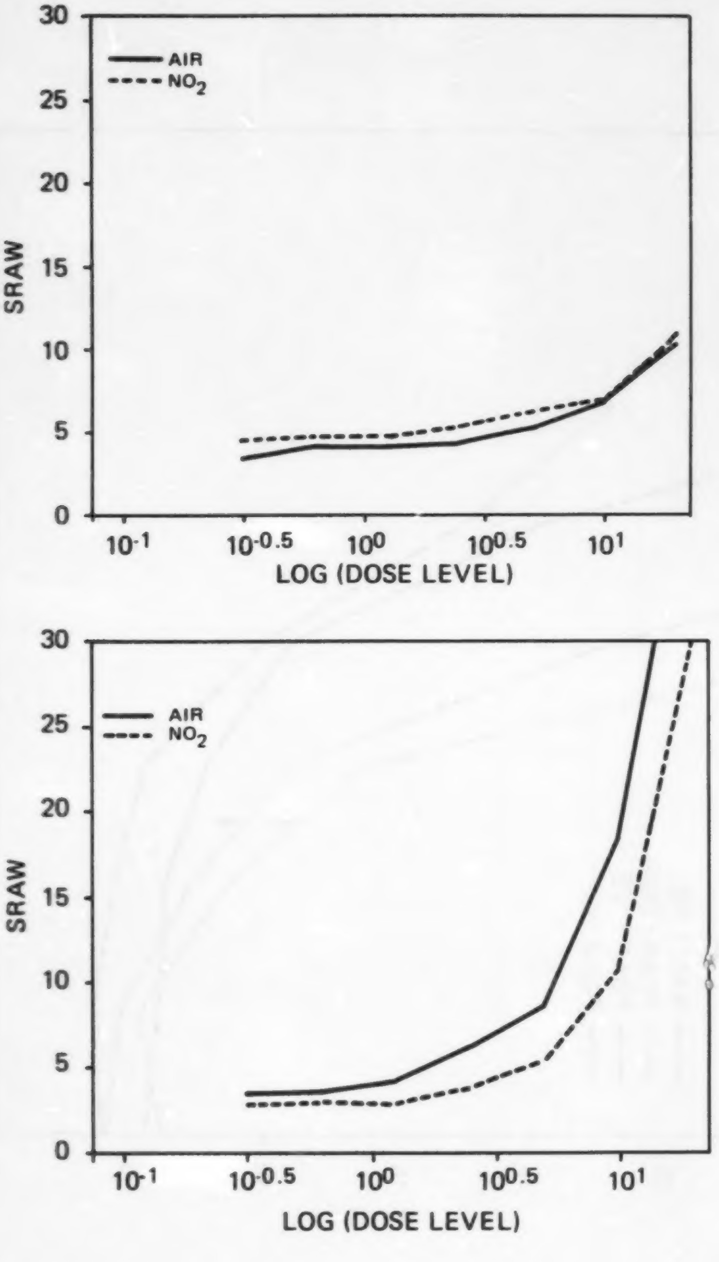

Figure 33-2. Specific airway resistance following two post-exposure bronchial challenges in two normal subjects exposed to air and NO_2.

have thresholds of >5 mg/ml. However, the slopes of the lines beyond the thresholds differ: the lower subject obviously shows a much increased slope. Other workers have suggested that these parameters (dose threshold and slope) characterize a given subject but do not necessarily correlate with one another; i.e., a high threshold is not always associated with a reduced slope, as demonstrated by the subject in the lower panel of Figure 33-2.

Another important aspect of Figure 33-2 is the apparent lack of pollutant effect on base-line SRAW.

Figure 33-3 (two asthmatic subjects) again depicts only the dose responses obtained immediately after air and NO_2, 1 week apart. These subjects display lower thresholds than the normals in Figure 33-2; in each case, the threshold is <1 mg/ml. Also, both have steep slopes.

The plots in Figure 33-3 (at least those in the lower panel) again suggest no significant pollutant effect on airway responsivity. There is some suggestion of a pollutant-associated shift to the right in the upper panel.

Figure 33-4 presents the full spectrum of seven dose responses obtained in the asthmatic subject of Figure 33-1. This subject's dose responses should probably be examined week by week by comparing, in this case, PDAR 4 to PDAR 1, both of which are pre-exposure dose-response curves. For the week of air exposures (upper panel), this subject's dose-response curves are for some reason shifted to the left of the base line. Therefore, the effect of

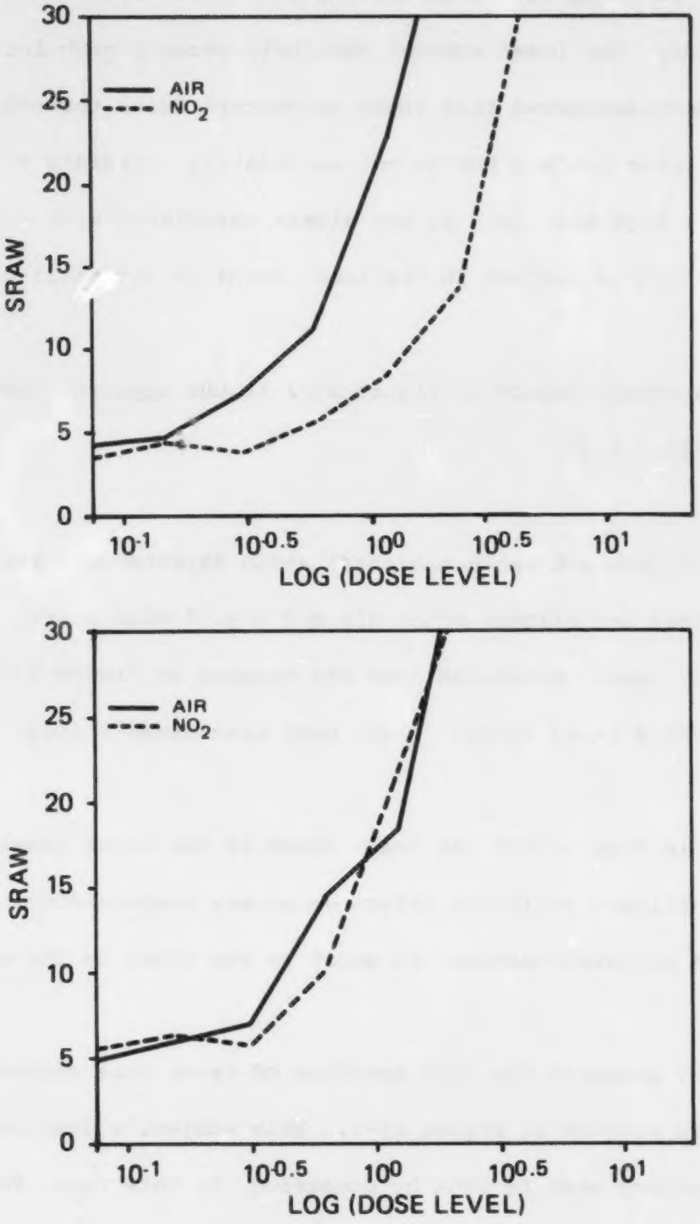

Figure 33-3. Specific airway resistance following two post-exposure bronchial challenges in two asthmatic subjects exposed to air and NO_2.

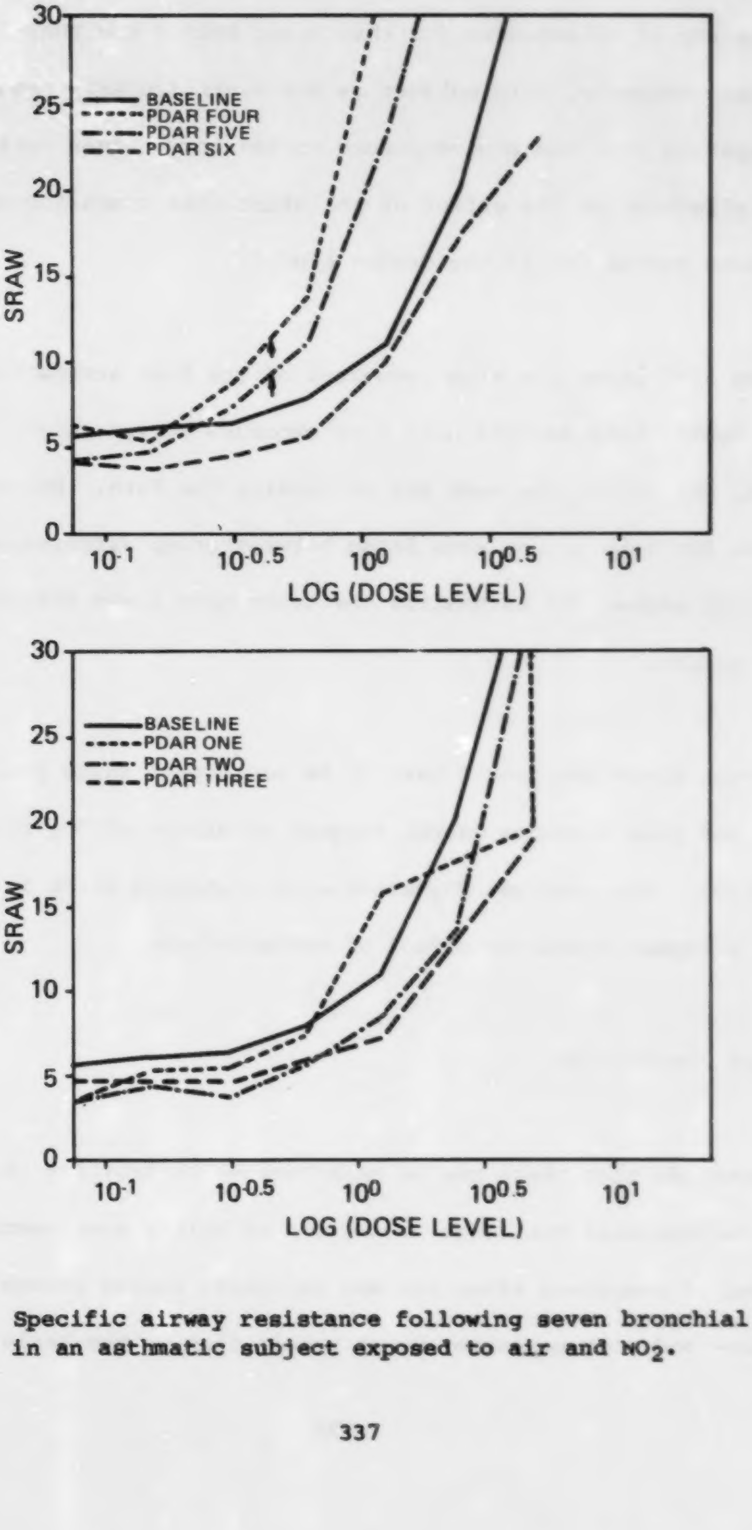

Figure 33-4. Specific airway resistance following seven bronchial challenges in an asthmatic subject exposed to air and NO_2.

pollutant is best examined by comparing bronchial challenges done 24 h before and immediately after exposure (in this case, PDAR 1 and PDAR 2 in the lower panel). Unfortunately, this subject is not a particularly good example. Still, comparing pre- and post-exposure curves for a given week probably gives a better reflection of the effect of pollutant than comparing only post-exposure curves (as in the Orehek study).

Figure 33-5 shows the mean responses of the four asthmatics and four normals. Again, this depicts only post-exposure curves which, as we have just discovered, may not be the best way to examine the data. Nevertheless, these mean values for both groups show large between-group differences in both threshold and slope; the asthmatics obviously have lower thresholds and increased slopes.

Although these data would have to be considered quite preliminary, the mean pre- and post-exposure values suggest no effect of NO_2 in either normals or asthmatics. The apparent NO_2-associated rightward shift in asthmatics may represent a repeat treatment effect of methacholine.

PRELIMINARY CONCLUSIONS

We conclude that there may be week-to-week variability in parameters of response to bronchial challenge in a group of mildly symptomatic asthmatics. Comparisons of responses after air and pollutant should probably be made between pre- and post-exposure curves rather than on the basis of

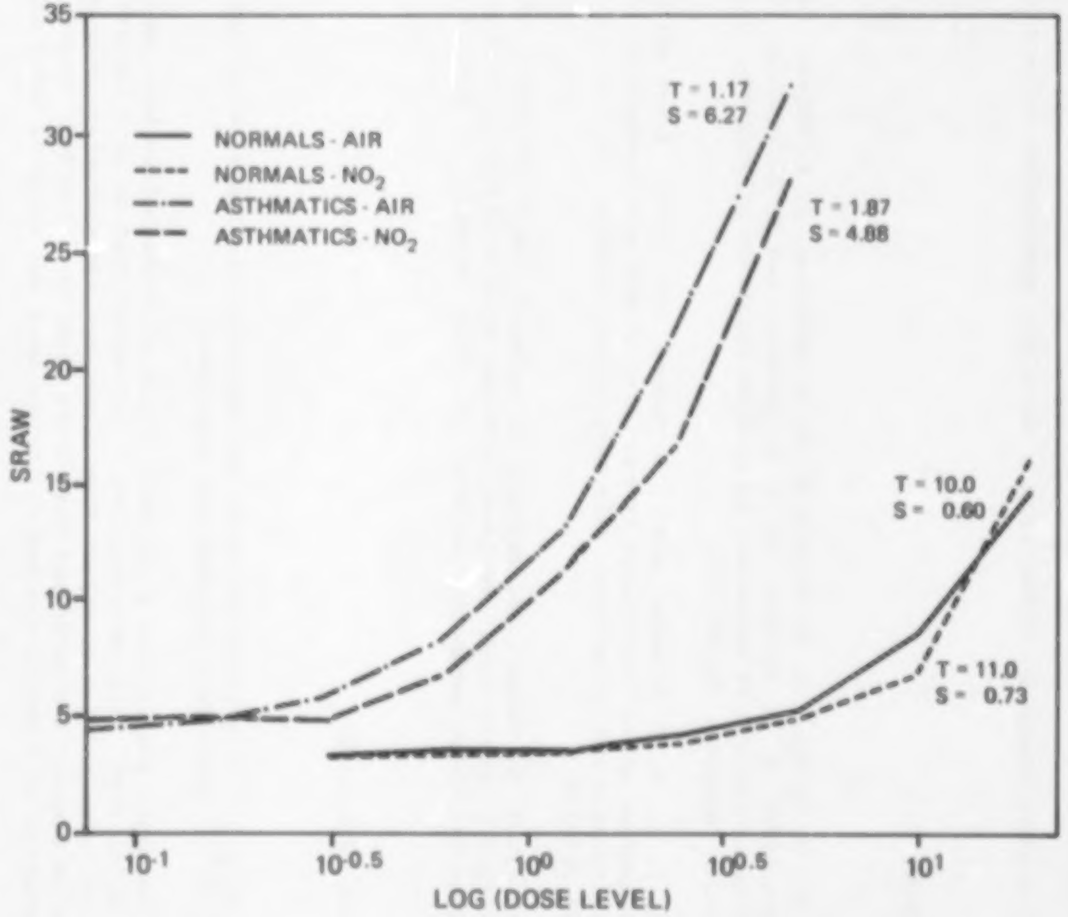

Figure 33-5. Mean specific airway resistance following two post-exposure bronchial challenges in four normal and four asthmatic subjects exposed to air and NO_2.

post-exposure curves only. A confounding variable in this type of analysis may be a repeat treatment effect of methacholine. Our preliminary data suggest that a 1-h exposure to 0.1 ppm NO_2 has no demonstrable effect on airway responsiveness in either normals or mildly symptomatic asthmatics.

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WORKSHOP COMMENTARY

P. E. Morrow: It's important to do the bronchial challenge before and after exposure; at least that has been our experience.

How might you explain a residual effect of methacholine that persists from day to day? With carbachol, which is established to be a longer-acting drug, we certainly see no effect from a previous administration even over a matter of hours. I'm surprised that there might be a residual effect from methacholine on a day-to-day basis. Do you have any thoughts about this?

J. F. Ginsberg: That's a good question. We really don't have an answer; we were equally surprised. We are currently looking at methacholine shelf life; the fact that we make up methacholine fresh daily may have something to do with the decreased responsiveness that we see. If there is some type of adaptation to methacholine, it may possibly be due to a longer persistence on receptors by more active metabolites.

34. HEALTH EFFECTS OF AIR POLLUTANTS IN THE TEXAS GULF COAST AREA

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INTRODUCTION

The 1977 Clean Air Act Amendments mandate that EPA perform a study of the nature, transport, and effects of pollutants in the Gulf Coast area. EPA's Health Effects Research Laboratory at Research Triangle Park, North Carolina is sponsoring the biomedical portion of this project; a companion effort by the Environmental Sciences Research Laboratory (Research Triangle Park) will examine pollutant sources and transport.

This report describes the planned epidemiologic studies, emphasizing the background work that led to their design. Also described are some of the various scientific "shoals" that we hope to avoid. No data are reported, for this project remains in the planning and coordination stages.

BACKGROUND

The specific congressional charge for this work occurs in Section 403(d) of the Clean Air Act Amendments of 1977:

> (d) The Administrator of the Environmental Protection Agency shall conduct a study of air quality in various areas throughout the country including the gulf coast region. Such study shall include analysis of liquid and solid aerosols and other fine particulate matter and the contribution of such substances to visibility and public health problems in such areas. For the purposes of this study, the Administrator shall use environmental health experts from the National Institutes of Health and other outside agencies and organizations.

Various legislative support documents prepared by relevant congressional committee staff broaden the scope of this effort to oxidants as well as aerosols. Background material also suggests that this work focus on the city of Houston, Texas.

We perceive a dominant attitude in the Houston area that it is unreasonable to develop national regulations from data that have been collected primarily in one geographic area. (With respect to oxidants, of course, this area is Southern California.) Although this attitude is sometimes expressed in a more heated than enlightening fashion, there is underlying validity in the complaint. As more studies are conducted and more data become available, it becomes very apparent that a high degree of circumspection is required whenever one generalizes results gathered in one area to other areas. Therefore, we believe that is is scientifically reasonable to undertake studies in the Texas Gulf Coast.

In view of these factors, we have resolved not to second-guess which pollutants to assess. Rather, we intend to determine the range of pollution exposure in the Gulf Coast, the concentrations, the temporal distributions, etc. before we irreversibly commit ourselves to embark on any specific study.

Since Congress appears to want a rather well defined package of studies, we feel that the project demands a certain balance between short- and long-term ambient pollution exposure. Similarly, we consider ourselves well advised to deal with a spectrum of health in the population (insofar as resources permit); specifically, we will examine individuals who are considered healthy as well as those who are sick. Finally, although the studies will be tailored to the local situation in the Texas Gulf Coast, we have selected study parameters similar to those already studied in Southern California. We thus hope to enable maximum comparability of results from the two geographic areas.

PLANNING STUDY

Our first effort towards fulfillment of the congressional charge was to enter into a contract with Radian Corporation (Texas) assisted by the Southwest Research Institute (Texas) for an assessment of the kinds of studies (in both the air quality and biomedical areas) that would be most useful. The biomedical portion of the resultant planning document recommended five or six different kinds of studies as "highest priority."

First, the planning document recommended that we assess the influence of local air pollution on the development of lung cancer in the area. We consider this to be a quite reasonable recommendation: over the past 15 years or so, lung cancer rates (at least for white males) have practically doubled in Harris County, the county in which Houston is located. The current annual incidence in white males is estimated at ~70 per 100,000 population, compared to a national incidence of ~40.

The planning document also recommended that we assess whether the local ambient air has any mutagenic potential. Because no actual human effects would be assessed, such a study does not seem particularly appropriate for the biomedical arm of this rather circumscribed congressional charge. Perhaps in connection with a study of cancer some mutagenicity assessment would be appropriate; opinion is divided on that point.

Thirdly, the planning document recommended that EPA perform a study of maximally exercising individuals who exercise repeatedly and in a standardized fashion. This seems to be a quite reasonable recommendation, and we have planned such a study. The background for this work comes from both experimental and epidemiologic studies. There is now a great deal of experimental evidence that oxidant (at least, ozone) effects become more and more discernible as the level of exercise increases. On the epidemiologic side, the very interesting study almost 15 years ago of high school cross-country runners in San Marino, California (Wayne et al. 1967) remains one of the most convincing of all air pollution epidemiologic studies. The Wayne et al. study has certainly been stressed in the preparation of oxidant criteria documents and standards. Therefore, a study of maximally exercising people fulfills several of our preliminary criteria.

Another recommendation of the planning document was that EPA undertake an ongoing health surveillance system, i.e., longitudinal tracking over several years of the occurrence of acute respiratory illness in children. We do not feel that this type of effort fits comfortably into the program that Congress intended: (1) Such a study is rather open-ended; (2) a careful study to relate disease incidence to local air pollution would of necessity be very time-consuming and very, very expensive (consuming all the resources that Congress appropriated for the entire effort).

Finally, the planning document recommended that EPA undertake serial studies of asthmatics, whose symptoms, physiology, and/or daily fluctuation in use of medication might be correlated to short-term ambient pollution exposure. We consider this to be an interesting recommendation, largely because there are again both experimental and epidemiologic underpinnings for the work. A great deal of the experimental underpinning comes from Dr. Jack Hackney, whose work with reactive and unreactive normal subjects as well as chronic asthmatics suggests that persons with reactive airways or wheezing tendency may be unusually sensitive to experimental and/or ambient oxidant exposures (Hackney et al. 1975a, 1975b; Linn et al. 1978). For reasons discussed below, we propose to do the asthma assessment not in children but in adult-onset asthmatics.

To summarize, the three recommended studies that EPA will most likely pursue are: (1) a study of lung cancer in the area; (2) a serial assessment of asthmatic symptoms, simple pulmonary physiology, and medication use; and (3) a serial assessment of the performance, physiology, and (probably) symptoms of people exercising vigorously in the field in a standardized and repeated fashion.

We originally contemplated a fourth study that was not specifically recommended by the planning document: a cross-sectional study in which questionnaires concerning persistent respiratory symptoms and relevant covariates would be distributed to adults in areas of differing pollution exposure. In EPA's hands, this general strategy has proven workable as well as useful from a regulatory standpoint. Previous cross-sectional studies on sulfur oxides and particulates have demonstrated an internal consistency of results (at least, qualitative consistency) that tends to reinforce the findings of other EPA studies. For the time being, limitations of financial resources and manpower preclude the inclusion of a cross-sectional questionnaire study in the Gulf Coast project. Should one of the three planned studies prove unfeasible, however, we would certainly reconsider this type of effort.

FEASIBILITY ASSESSMENT

Prior to collecting any data, we intend to complete a thorough feasibility ascertainment for each of the three planned studies. Toward this end (and toward scientific assistance throughout the remainder of the project), we are assembling an extramural advisory group that will formally convene from time to time, and with whose members the investigators will maintain frequent informal contact. The disciplines to be represented in the group are epidemiology, biostatistics, air quality measurement, historical estimation of air quality, pulmonary medicine and physiology, and clinical allergy. Hopefully, the advisors will help us avoid any severe methodologic or interpretive problems.

Lung Cancer Study

The proposed study of lung cancer will receive extensive feasibility assessment. The venue for the proposed effort is Harris County, Texas, the county in which Houston is located. Currently, 700 to 750 new cases of lung cancer are estimated to occur in Harris County each year, the majority in white males. Female rates are much lower (~15 per 100,000 population). However, we may be well advised to examine females, since they may be less likely to undergo occupational exposure in the petroleum and petrochemical industries.

A few years ago, MacDonald (1976) geographically charted the incidence of lung cancer in Harris County. She found a correspondence between concentration of incidence and either (1) proximity to the Houston Ship Channel (a petroleum and petrochemical industrial hub) or (2) location in a downwind path from the Channel. However, Marmor (1978) pointed out that MacDonald did not have the opportunity to measure and assess socioeconomic and occupational variables. She did not quantitate smoking differences, nor did she specifically consider population mobility (certainly a crucial factor in rapidly growing Harris County).

Henderson and associates recently completed a case-control study of lung cancer in Los Angeles (Pike et al. 1979). Preliminary observations (Menck et al. 1974) resembled those by MacDonald: lung cancer cases appeared to cluster in an industrial area of the Los Angeles region. Following a more careful case control study, however, all of the discernible effects in this geographic area were observed to result from differential occupational exposure. Henderson was unable to turn up any effects resulting from ambient exposure alone.

Our feasibility effort for the cancer study will be complex, particularly with respect to air quality estimation extending back over the past few decades. We hope to be able to geographically characterize the county in terms of high, intermediate, and low pollution classifications for individual pollutants and for combinations of pollutants. We plan to assort cases and comparison subjects into these zones and then, after adjusting for appropriate covariates, determine whether the proportion of cases in high pollution areas differs from the proportion in the control group. Obviously, the success of this case-control study will directly depend on the confidence with which we can construct past exposure estimates for Harris County. Should reconstruction prove unsuccessful, we will have to choose between adapting the study design or abandoning the study altogether.

Another important feature of the lung cancer feasibility study will be to ascertain the potential cooperation of the local medical community. The study cannot succeed in the absence of very good relations with the pulmonary clinicians in the area.

Our feasibility study will also seek to evaluate the existing tumor registry capacity. How quickly will we be informed when a new lung cancer case occurs? A lack of rapid turnaround would require us to build our own surveillance system into the study. Some well established tumor registries take, at best, ~12 to 18 mo to inform an investigator of a specific new case. For lung cancer, this is far too slow: the 1-yr mortality for newly diagnosed cases of lung cancer is ~85%.

In summary, successful completion of the lung cancer feasibility study--particularly the estimation of air quality for previous years--will be a considerable task both scientifically and politically. In our opinion, however, the rise in lung cancer rates as well as their current absolute magnitude justify this effort.

TENTATIVE PROTOCOL

Performance of the feasibility and full-scale studies will proceed under a cooperative agreement executed in late 1979 between EPA and the University of Texas School of Public Health at Houston. Principal investigators from the University of Texas will be Dr. Patricia Buffler and Dr. Reuel Stallones, Dean of the School of Public Health. This author will serve as principal investigator (biomedical) for EPA. All parties anticipate the effort to be a cooperative agreement in more than name only.

Lung Cancer Study

Should our feasibility efforts predict favorably for success of the lung cancer study, we plan to collect data from cases and comparison subjects over the entire three-year period of 1981-1983. If the whole project succeeds, a final report will be issued by mid-1984.

Asthma Study

Our ultimate goal in the asthma study will be to follow ~60 adult-onset asthmatics on a daily basis for 6 mo. We plan to distribute to these subjects some device by which each can easily measure one or more parameters of lung function each study day. The device used may be a Mini Wright Peak Flow Meter or perhaps an instrument such as the Vitalor, which yields a permanent tracing of the forced vital capacity maneuver.

Our reason for concentrating on adult-onset asthmatics is that we want to consider the important covariate of fluctuation in medication use. To date, this covariate has not been formally assessed in serial studies of asthmatics; therefore, available studies are open to considerable questions of interpretation. Warnings to doctors about the dangers of nebulizer use in children have appeared in the pediatric literature. Some sobering stories of nebulizer overdose and paradoxical nebulizer effects in children have convinced us that childhood asthma, despite its theoretical advantages, may not be an appropriate field of study in this project. We welcome further comment on the relative appropriateness of childhood and adult asthma.

The "nebulizer chronolog"--an instrument to measure nebulizer use--is not fully tested but may be useable by the time our study starts (probably in the spring of 1981). The nebulizer chronolog records the time of day at which each squirt of the nebulizer occurs; thus, we will have at least the daily number of squirts (although it's unlikely that we'll be able to tell whether one squirt is as big as another).

Assuming the feasibility study forecasts success for the asthma project, we expect to issue a report by mid-1982.

Exercise Study

With respect to the exercise study, it is not possible in this report to outline a proposed schedule. The schedule will depend integrally on the number of subjects that are ultimately selected, on their frequency of standardized exercise, on the range of pollution exposure at the study site, and on the complexity of that exposure.

In this author's opinion, the most desirable subjects would be "dedicated adult joggers" who strive to exercise vigorously each and every day, who run a standard distance, and who perhaps even try to improve their time each day. It is not certain that we can obtain such a group, largely because of the absolute requirement for standardized exercise on every occasion. Perhaps no informal group of adult joggers, regardless of personal dedication, would fulfill this requirement. For this reason, we will canvass the area not only for adult subject groups but also for possible high school and college groups. We have already determined (to our dismay) that the high school cross-country schedule will not permit replication of the Wayne et al. (1967) study mentioned earlier. This is because high school cross-country meets tend to occur at different locations within cities and even within tri-county areas. Such constant changes of exercise location might exert important unmeasured effects on the results.

We hope to produce our report on the exercise study shortly after the report on asthma (i.e., mid-1982 or perhaps a little later).

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WORKSHOP COMMENTARY

Comment: We have heard a lot [at the Research Planning Workshop on Health Effects of Oxidants] about the animal infectivity model. I haven't heard you describe any planned studies to investigate human susceptibility to infection.

Are you planning any, or is that something that will be available only from animal toxicology?

R. S. Chapman: I'd love to do a study like that on a community level. But, frankly, I'm not sure that it's doable right now. An epidemiologic study dealing with pollution and respiratory infection would require careful assessment of specific etiologic agents over time; this would be a very difficult prospect in itself. It would require a group of unusually willing subjects, because (in my opinion) one would have to do serologic testing at a fairly short interval. For instance, there's evidence now that the immune period for respiratory syncytial virus in children may be as short as 3 mo. This means at least a couple of bleedings per year--a rather ticklish prospect on an epidemiologic plane. I'm disheartened to say, though, that a bigger deterrent is the fact that such a study would need to be long-term (a decade of data collection, at least). Frankly, I don't think the present EPA funding structure could realistically absorb such a long-term study.

Comment: We see so much of these infectivity models in terms of the criteria document; I would think it would be important to--

R. S. Chapman: I agree with you one hundred percent. There have been some indirect assessments along this line in self-administered cross-sectional questionnaire studies in which mothers were asked whether children had certain specific syndromes diagnosed by a doctor over a given period of time. Because they rely on the mother's recall, such studies aren't (theoretically, anyway) ideal. More prospective assessments of acute respiratory illness in all family members have been and are being performed by EPA; Lee [Chapter 30 of this volume] mentioned one such study. These studies allow one to compare communities with respect to gross incidence of upper and/or lower respiratory illness, perhaps with an allergic overtone. I don't think they allow the analysis to become any more specific than that. Since these studies are performed on a daily basis, time series statistics might conceivably be developed to allow some shorter-term inference. However, I feel that including some assessment of community experience with specific etiologic agents would be a highly desirable adjunct to this kind of work.

Question: With respect to your asthmatic pulmonary function study, will you draw upon the data that were collected in the Houston Area Oxidants Study?

R. S. Chapman: Those data may be helpful in locating potential subjects. Before we start, we hope to know where virtually every adult-onset asthmatic in the area lives so that we can focus on a couple of residential clusters (ideally, just one cluster), in order to minimize our aerometric requirements for ambient measurement. Other than that and some of the initial recruitment methods, I'm not sure that the Houston Area Oxidants Study data will be particularly useful to us. If those data are carefully reanalyzed, their usefulness may increase.

Comment: Everything you've described has involved human pulmonary effects or animal experimentation that includes extrapulmonary effects. Do you plan to look at any human extrapulmonary effects?

R. S. Chapman: The only possible nonpulmonary work would be some simple cardiovascular physiological measurements (perhaps heart rate, blood pressure, and/or electrocardiogram) on subjects in the exercise study.

Question: What about blood chemistry?

R. S. Chapman: Probably not, although the door isn't entirely closed. We must make certain decisions with respect to our financial resources. For one thing, we won't be able to rely on ambient measurements alone; we will probably have to do some indoor measurements before we're through. (Whether those are performed in the homes of subjects or in similar homes nearby has not been decided. We're asking a lot of the subjects already.) Blood chemistries, desirable as they might be, probably won't be possible just because of resource limitations.

Question: Was Houston involved in the third National Cancer Survey?

Comment: Yes.

Question: Have you compared Houston with other cities with respect to lung cancer? Was it always higher?

R. S. Chapman: No; of the six-county area, Harris County has the second highest incidence rate. Galveston has a somewhat higher rate.

Comment: I was more interested in a comparison to the other cities examined in the National Cancer Survey.

[Comments inaudible]

R. S. Chapman: One of the reasons we have to find some relatively low exposure areas within Harris County is to provide grounds on which to assess pollution effects. If there is homogeneous exposure throughout the county, there's no point in doing a case-control study.

Some of the available data (admittedly, incomplete) suggest that there is some kind of exposure gradient over the years. Whether we can estimate that gradient is another question entirely.

Question: Do I understand correctly that you intend, in the exercise study, to obtain hourly values for sulfate and nitrate? Can that be done?

R. S. Chapman: If we gave you that impression, we either weren't thinking very clearly or didn't express ourselves very clearly in writing. I don't see any theoretical deterrent to gathering hourly filter samples for those substances. I don't see much point in going finer than that; hourly values themselves may be difficult to obtain. I'd appreciate comment on this point. Do hourly measurements of nitrate make any sense? I think they're doable, but do they make any sense? Is the distortion-to-real-information ratio so high that the hourly measurement is going to be "garbage"? Or does the ratio stay constant regardless of the sampling interval?

Comment: It's a variable measurement demanding care in technique.

J. D. Hackney: Do I understand correctly that you intend to study adult-onset asthmatics?

R. S. Chapman: Yes, but I can be talked out of it! I have a nebulous suspicion that there may be something different about adult-onset asthmatics in comparison to childhood-onset asthmatics who remain asthmatic as adults. I have no concrete evidence. Do you think that my concern holds any water at all?

J. D. Hackney: I just wanted clarification.

Question: Are you speculating that the adult-onset asthmatic is less likely to be an atopic asthmatic and more likely to be a perennial nonseasonal asthmatic?

R. S. Chapman: In all candor, my thinking wasn't that refined. It is highly unlikely that we will have more than 60 subjects stay with us throughout the course of this work. Therefore, we might be well advised to impose a certain homogeneity on the group. I could certainly be convinced that it would be best to choose adult asthmatics with childhood onset rather than adult-onset asthmatics. On the other hand, if there's absolutely no reason for that, we could certainly widen the field of acceptance.

Question: In the exercise study, how do you plan to perform all the pulmonary function assessments "at once" (when they all "pile up on you")?

R. S. Chapman: That's a good question--one that we haven't really worked out yet. The number of testing technicians ultimately hired will probably depend largely on the number of subjects. My own feeling is that no single group will contain more than ~15 subjects. It seems to me that three testing technicians could test this number of people spirometrically in a rather short period of time. I would not expect a single subject (especially a trained one) to stay with the testing technician for more than 3 min.

But another question arises: Would we be well advised to forget about testing them immediately post-exercise? Would we be smarter to test them, say, 20 min post-exercise after they've had a shower? That would give us a standardized interval for testing; testing immediately post-exercise, especially after an exhausting routine, might not be very informative.

M. Goldman: With respect to the lung cancer study, isn't change in population makeup a crucial facet? There is probably a 20- to 30-year latent period for lung cancer. Therefore, if there is an environmental association, those at risk may have received it at another location. Secondly, it's very difficult to remove the overwhelming effect of cigarette smoking both in women and in men. If we do this, there may be very little left. This is especially true when we try to determine what the "petrochemical" environment was 20 years ago and what it is at this time. In sum, are the problems of an intracounty assessment surmountable?

R. S. Chapman: That is a succinct statement of our own concern.

35. OVERVIEW OF RESEARCH AND REGULATORY ACTIVITIES OF THE CALIFORNIA AIR RESOURCES BOARD

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INTRODUCTION

This report surveys the research and regulatory functions of the Air Resources Board (ARB) of the State of California. Chapters 36 through 39 of this volume present details of some of the oxidant health effects studies.

BACKGROUND

The California State Government has sponsored air pollution research on a substantial scale for the past 10 years or so. This author heads a program which actually began in the University of California as Project Clean Air. About 1970, that program was shifted from the University to ARB and expanded to include investigators from outside the University of California system.

Our charge is to do applied research on problems that are unique to and critical in California. Over the years, we have tried to adhere fairly closely to that mandate. The program was developed in much the same way that EPA's program is now being developed: client relationships were established with all the various ARB divisions and branches. Input from these clients allows us to propose, on a yearly basis, a research program that responds to their needs.

Year-to-year variability in the funding level sometimes makes it difficult to plan with certainty. Funding has ranged from ~$1.5 to ~$3.5 million a year. We operate on a zero-base arrangement: each year ARB puts together a set of projects and takes it through the budget process from the Governor's Office to the Legislature. Appropriation is on a project-by-project basis.

For the budget year beginning in July 1980, we hope to have ~$4 million for research within the State of California. The Governor has proposed a fairly healthy increase for us this year; what the Legislature will do with this proposal remains to be seen.

RESEARCH PROGRAM

The ARB research program is divided into seven different categories; each is discussed below. Most of the reports originating from this program are available through the National Technical Information Service in Washington, D.C. Available from our office in Sacramento is a short report containing one-page summaries of all ARB projects conducted over the past two years.

Effects of Air Pollution

Research into the effects of air pollution represents a major part of our program. Health effects research has accounted for ~20% to ~25% of the total research budget over the past five years; the fraction allocated to health effects research in the proposed 1980 budget is somewhat larger. A number of problems remain to be resolved, and ARB will need better and better information to establish California air quality standards in the years ahead.

The other important area is research into effects on vegetation; ~10% of our budget is devoted to this category. Agriculture is the State's largest industry; the value of crops produced in 1978 was ~$12.5 billion, with another $2 billion generated by the forest products industry. Thus, the State of California is very concerned about limiting damage to crops and forests.

Economic Impacts of Air Pollution Control

In the last couple of years, ARB has become very involved in estimating the economic impacts of control options. One of our contractors is working to incorporate air pollution control in a statewide input-output economic model. This model is designed to consider economics at the stage when control measures are first proposed. When the model is up and running, we'll be able to test various proposed control measures for economic impacts on directly affected industries. Beyond that, the model will permit us to determine how the costs of air pollution control filter down through our State's economy.

Emissions Inventory and Control Technology

The third category of our research program--emissions inventory and control technology--receives the largest fraction of funding. About 40% of our budget is devoted to this research, which includes studies to improve emissions inventories for stationary sources and cars, feasibility studies on the kinds of control equipment that might be adopted for use in California (e.g., scrubbers and ammonia injection as a means to control nitrogen oxides emissions from large sources), and so on.

Atmospheric Processes

About 15% of our budget is committed to a catchall category called "atmospheric processes." We've sponsored a great deal of smog chamber work by James Pitts' laboratory at the University of California - Riverside. These studies are designed to sort out some of the intricacies of photochemical smog formation and to determine the fates of various organic molecules present in the air (solvent hydrocarbons, pesticide hydrocarbons, etc.).

Also funded in this category is a great deal of work in the field. For example, ARB sponsors tracer studies and airborne measurements to establish source-receptor relationships and track pollutant movements between the State's various air basins or air quality control regions. Results from this work are calling into question a fundamental assumption of our State law--the assumption that the State can be divided into separate and independent air basins or sheds. We now know that a great deal of pollution is transported from such locales as the San Francisco Bay Area to the Great Central Valley, and from the Los Angeles area down to San Diego and out into desert areas. So, what happens in the way of controls upwind is a very important determinant of what happens in air basins downwind. Our field studies establish these relationships.

Air Quality Modeling

The fifth category, air quality modeling, entails the development of models that simulate emissions and such atmospheric processes as chemical transformation and dispersion. There are regional models in various stages of development for San Diego, Los Angeles, Sacramento, and Fresno. The Bay Area model was developed independently of our support.

Although air quality models seem to be the wave of the future, our experience shows them to be a mixed blessing. In principle, such models can provide very precise information on the benefits of various control measures. But air quality models are also "data hungry": To exercise a model properly and with confidence requires a great deal of air quality data, meteorological data, emissions data, and so forth. Thus, although ARB is making progress in this area, we have also discovered that it takes longer than expected to reduce theory to practice.

Meteorological Forecasting

Because air pollution can reach very high levels in such areas as Los Angeles and the Central Valley, meteorological forecasting is an important part of our research program. We need to be able to forecast the occurrence of severe episodes of photochemical smog in Los Angeles and of the trapping of smoke from agricultural burning in the Valley during winter months. When employed by research meteorologists, advanced statistical techniques can be used to very handily forecast the occurrence of such emergencies at least 24 and possibly as long as 36 hours in advance.

Measurement and Measurement Methods

Finally, ARB sponsors a fair amount of work on air pollution measurement and measurement methods. These efforts focus on ambient levels. For example, we've worked with the California Institute of Technology and with the University of California - Berkeley to develop methods to survey acid precipitation. A report on acid rain in California will be issued within the next two months.

The laboratory of James Pitts at the University of California - Riverside has developed sophisticated measurement techniques for vapor-phase ammonia, a compound that is very important in the formation of photochemical aerosols due to its neutralization of sulfuric and nitric acids. Understanding ammonia is important to understanding the nature of the particulate burden in many areas of the State as well as for dealing with the problem of visibility degradation.

CALIFORNIA AIR POLLUTION STANDARDS

For more than a decade, the State of California has promulgated its own ambient air quality standards and other standards to limit community exposure to various pollutants and combinations of pollutants. These California standards fall into three distinct categories: ambient air quality standards, alert levels, and standards for hazardous substances.

Ambient air quality standards are, of course, levels that are considered to be safe over the long term for the general population.

Alert levels are specific ambient levels at which extraordinary measures are taken. For example, the State has established alert levels for ozone that require special action on the part of industry and, in some cases, the population in general. The best known is the school alert level for ozone: when ozone rises to 0.20 ppm and that concentration is expected to persist for an hour or more, schools and colleges are notified. Most of our medical advisors believe that children, especially, should curtail their outdoor physical activities when ozone reaches this level.

Recently the State has considered standards for hazardous substances. This is a relatively new area for the Federal Government as well as for us.

These hazardous substance standards are somewhat different from our ambient standards or alert levels in that they are targeted specifically at the vicinities of sources (factories, etc.). For example, about two years ago ARB adopted a hazardous substance standard for vinyl chloride. In California, vinyl chloride is not a ubiquitous pollutant--it's localized to three or four individual sources in the Los Angeles Basin. Our standard provides that vinyl chloride levels will be monitored in the vicinity of each plant (i.e., at the property line or thereabouts). The rationale for this approach is that most of the emissions from such facilities do not emanate from a stack or any particular large source; rather, they are "fugitive emissions" (principally, leaks and accidental releases). It is very difficult to write an emissions regulation that addresses fugitive emissions in an effective way, so ARB adopted an air quality standard for the vicinity of each vinyl chloride facility. Thus, each facility is free to figure out the best way to reduce its own emissions to a level that is safe for the surrounding community. This makes the job of ARB easier, too: it's simpler to write this type of regulation as compared to a detailed engineering specification type of regulation.

The ARB procedure for adopting standards is somewhat different (and in some ways simpler) than EPA's procedure. Responsibility is divided between ARB, which actually adopts the standard, and the State Health Department, which is required under State law to advise ARB on health-related standards. The State Health Department, in turn, is advised by a committee of physicians appointed by the California Medical Association.

In contrast to the private or closed-door method of EPA (in which the Administrator proposes standards on the basis of staff recommendations), California standards are adopted via a public hearing process. In this author's opinion, there are virtues to both systems. In the California system, the ARB staff does a great deal of the groundwork prior to the hearings, and the hearings can be lengthy. However, the Board itself--the group of individuals who must ultimately make the decisions--is exposed to a much wider range of viewpoints than in the Federal system.

Our system also differs from the Federal system in that there are no rigid timetables for attainment of standards. The ARB views standards more as ultimate objectives of the air pollution control program. The State statutes do require "reasonable progress" toward attainment of standards, but there are no rigid timetables. As technology develops and as economic conditions permit, ARB and the local air pollution control officials implement emission controls to move ahead toward our standards. This gives us a fair degree of flexibility that is not present in the Federal system.

In California law there are no distinctions between primary or health-related standards and secondary or welfare-related standards. Standards adopted by ARB are assumed to be protective of both human health and welfare. There are only a couple of standards that are strictly welfare-related: the standard for the hydrocarbon ethylene (a specific toxicant for certain kinds of plants), and the State visibility standard, which has turned out to be our most stringent standard, the toughest to meet, and the one that we've made least progress toward attaining.

WORKSHOP COMMENTARY

Question: Do the budgetary numbers that you quoted represent the budget of your research program, or is that the total budget of ARB (which, I presume, involves a great deal of expenditure for administrative costs and all the noninvestigative functions)?

J. R. Holmes: Those figures are for actual extramural research projects. Those are the dollars that we "shovel out the door," so to speak.

Question: How does the yearly uncertainty in funding levels affect your ability to get other people to do work related to ARB goals?

J. R. Holmes: Well, it's certainly a problem. Some of the projects that we would like to do almost require a guarantee of continued funding (for example, prospective epidemiologic work). We've talked to the Legislature staff and tried to convince them that we ought to have this sort of funding capability, but so far we haven't met with success. The Legislators simply don't want to commit funds over that period of time.

36. LUNG INJURY AND DEPLETION IN THE MOUSE FOLLOWING EXPOSURE TO NITROGEN DIOXIDE

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INTRODUCTION

This report summarizes some preliminary animal experimental data which we consider highly relevant to the question of human adverse health effects resulting from exposure to oxidants. Lung tissue of mice exposed to nitrogen dioxide (NO_2) was analyzed for hyperplasia of Type II cells and protein leakage.

BACKGROUND

Preliminary studies of human lungs from the Los Angeles Medical Examiner-Coroner's Office and the Los Angeles County-University of Southern California Medical Center suggest that a progressive depletion of lung tissue occurs in everyone. The rate at which this occurs and the extent of the depletion in the well population are presently unknown. However, the crude indicators available (in particular, "naked eye" measurements of emphysema, and the frequency of bronchiolitis and structural disruption by microscopic study) point to a serious underestimation of the extent of destructive lung disease in the general population.

The underestimation of lung disease is largely due to the tremendous amount of lung reserves (e.g., ~50 billion Type II cells and ~30 billion Type I cells in ~300 million alveoli). Substantial numbers of these lung cells can be lost in the absence of overt clinical symptoms or signs, and routine pulmonary function tests may not become clearly positive until as much as half of the lung has been irreversibly altered or destroyed. Routine autopsies are not much more accurate: criteria for recognition and quantitation of the various kinds of emphysema fall far short of a uniform definition and application, and this affects most of the autopsies carried out in major universities and hospitals.

In essence, the major problem of air pollution is a disturbance in the microecology of the human body, i.e., the diverse cell societies not only of the lung but of other organs as well. As with the visible ecology, vanishing species of life is an early and serious part of the adverse effect. With respect to the lung, the Type I cell is of special concern: the ultrathin lining it provides for the alveoli is critical to gas exchange. Thus, the first target of our research effort was a quantitation or inventory of the Type I cell population.

METHODS AND RESULTS

Inventory of Type I Cell Population

The methodology we used to count Type I cells is an indirect one, based on the now well established fact that loss of Type I cells is manifested by a replacement or hyperplasia of Type II cells. First, we developed a means to single out the Type II cell for quantitation (Sherwin et al. 1967). Subsequently, mouse whole lung sections were processed for lactate dehydrogenase (LDH) responsiveness. We found LDH positive granules of the Type II cell to be densely packed around the nucleus, whereas LDH positive granules of macrophages and other lung cells were widely dispersed throughout the cytoplasm and relatively weakly stained. It should be noted that intra-alveolar macrophages are, for the most part, removed by frozen section processing procedure (i.e., intra-alveolar gelatin from perfusion-inflation is washed out during processing and carries macrophages with it). Furthermore, interstitial macrophages are not detected by the gray value setting since they have extremely thin and elongated cytoplasmic processes within the interstitium, and this minimizes the LDH response.

An image analyzer with a highly sophisticated detection system and field editor provided a very practical means to obtain accurate large-volume measurements not only of the number of Type II cells but of their sizes as well (Margolick et al. 1973; Sherwin et al. 1973). The number of Type II cells was adjusted to a base line of number of alveoli as reflected in the amount of alveolar wall area detected in the same field by the image analyzer. Additional measurements obtained at the same time were: numbers of Type II pneumocytes according to size distributions (i.e., by diameters of >8 µm, >10 µm, and >12 µm), internal surface area of the alveoli, and linear intercepts of the alveolar walls. Computer processing of the data permitted large-scale statistical analysis and diverse kinds of data analysis (Sherwin et al. 1979a). Macrophage quantitation and total alveolar counts are currently in progress.

Most recently, we studied the effects of 0.34 ± 0.02 ppm NO_2 on Swiss-Webster adult male mice. A total of 120 mice were equally divided into control and exposed groups. Exposed animals received NO_2 for 7 h/d, 5 d/week, for 6 weeks. The lungs of these animals were inflated with gelatin to approximate the volume of full lung expansion, and frozen sections were processed for the LDH reaction. Eight whole lung sections were obtained from the left lung of each animal and numbered according to location in a sagittal plane running medially (from the lung hilum) to the lateral lung peripheral area. Four fields were quantitated for each lung section, for a total of 32 fields per animal. The data obtained are summarized in the tables that follow.

Table 36-1 displays the data from each field treated independently and analyzed. The findings are:

TABLE 36-1. LUNG TISSUE DATA (FIELDS ANALYZED SEPARATELY) FOR MICE EXPOSED TO NITROGEN DIOXIDE VS. AIR

Exposure	Mean	Standard Deviation	Standard Error	T Value	Frequency Distribution	Probability (two-tailed)[a]
Number of Type II Cells (>8 μm)[b]						
NO_2	314.35	145.46	3.58	5.39	3211	$p \to 0$
air	288.05	130.04	3.30			
Area of Type II Cells (>10 μm)						
NO_2	3129.09	2885.15	71.01	4.42	3210	$p \to 0$
air	2722.58	2265.38	57.34			
Alveolar Wall Area (no sizing) Minus Area of Type II Cells[c]						
NO_2	39166.40	21265.98	523.37	1.48	3209	0.14(NS)
air	38044.79	21759.30	550.91			
Alveolar Internal Surface Area						
NO_2	2747.92	1231.38	30.30	2.95	3211	0.003
air	2622.36	1180.57	29.88			
Linear Intercepts of Alveolar Walls						
NO_2	8229.40	3749.41	92.25	3.45	3210	0.001
air	7786.04	3526.56	89.29			
Alveolar Wall Area (>10 μm) ÷ Number of Type II Cells (>10 μm)[d]						
NO_2	82.84	28.82	0.71	-5.16	3207	$p \to 0$
air	88.61	34.43	2.87			
Type II Cell Area ÷ Number of Type II Cells						
NO_2	10.91	4.94	0.12	1.70	3206	0.09
air	10.64	3.99	0.10			
Internal Surface Area						
NO_2	6.88	0.96	0.02	0.54	3210	0.59
air	6.86	1.04	0.03			

[a] NS = not significant.

[b] Per lung field.

[c] For wall area with sizing of >10 μm minus area of Type II cells (>10 μm), p = 0.441 (NS).

[d] Alveolar wall area ÷ number of Type II cells = inverse of number of Type II cells per lung field adjusted to amount of lung tissue (or number of alveoli); 1 unit = 3.2 μm^2. All combinations of two wall areas divided by three Type II cell measurements (>8 μm, >10 μm, >12 μm) provided p values → 0. All Type II cell measurements (>8 μm, >10 μm, >12 μm) provided p values → 0.

1. The numbers of Type II cells (>8 µm, >10 µm, and >12 µm in diameter) were increased for the exposed group ($p \rightarrow 0$).
2. The mean areas per field of the Type II cells were greater for the exposed group ($p \rightarrow 0$).
3. The value, alveolar wall area ÷ number of Type II cells per field, was greater for the control animals ($p \rightarrow 0$), and indicated a a greater number of Type II cells for the exposed animals since this is an inverse of the number function. Also, the wall area base line was not significantly different between groups, indicating that the ratio difference is a function of the number of Type II cells.

Table 36-2 displays the results of a two-way nested analysis based on 60 pairs of animals using a mean field value for each animal. We found an increase in Type II cells for the exposed group, but this increase was not statistically significant, apparently because of the great variability between animals. Also, the analysis assumed a normal distribution (e.g., no high responders).

Again using animal rather than field comparisons, we ranked the animals according to upper quartile distribution and subjected the ranking to chi-square analysis (Table 36-3). A statistically significant ($p < 0.025$) difference in the number of Type II cells was noted when the number of Type II cells was adjusted for alveolar wall area. (This adjustment compensates for variations of inflation of the lung during processing.) Without the adjustment, the difference was still apparent but only at the borderline of statistical significance ($p < 0.1$).

TABLE 36-2. LUNG TISSUE DATA (TWO-WAY NESTED ANALYSIS) FOR MICE EXPOSED TO NITROGEN DIOXIDE VS. AIR[a]

Measure		Distribution Frequency	Mean Square	Frequency Ratio	Probability[b]
Number of Type II Cells (>8 μm)	A(G)	112	327,573	32.96	$p \to 0$
	G	1	323,856	0.99	NS
	Residual	3067	9,939		
Number of Type II Cells (>12 μm)	A(G)	112	169,835	32.99	$p \to 0$
	G	1	122,024	0.72	NS
	Residual	3067	5,148		
Number of Type II Cells (>10 μm)	A(G)	112	230,859	32.51	$p \to 0$
	G	1	217,392	0.94	NS
	Residual	3067	7,101		
Type II Cell Area	A(G)	112	107,837,392	33.25	$p \to 0$
	G	1	91,516,928	0.85	NS
	Residual	3067	3,242,773		
Alveolar Wall Area	A(G)	112	10,420,183,040	51.50	$p \to 0$
	G	1	58,261,504	0.06	NS
	Residual	3067	202,329,168		
Alveolar Wall Area Type II Cell Area	A(G)	112	4,300,115,968	48.17	$p \to 0$
	G	1	26,804,224	0.01	NS
	Residual	3067	89,261,632		
Alveolar Internal Surface Area	A(G)	112	3,270,255,616	47.24	$p \to 0$
	G	1	675,282,944	0.21	NS
	Residual	3067	69,220,512		

[a] Animal nested within group; slide and field are considered replicates.

[b] NS = not significant.

TABLE 36-3. LUNG TISSUE DATA (CHI-SQUARE ANALYSIS OF CONTINGENCY TABLES) FOR MICE EXPOSED TO NITROGEN DIOXIDE VS. AIR[a]

Number of Type II Cells (<8 μm)

	L	U	Total
NO_2	38	19	57
air	47	10	57
Total	85	29	114

$X_1^2 = 3.75$ ($0.05 < p < 0.1$)[b]

Area of Type II Cells

	L	U	Total
NO_2	37	18	57
air	46	11	57
Total	83	29	112

$X_1^2 = 2.63$ (NS)

Alveolar Wall Area

	L	U	Total
NO_2	44	13	57
air	41	16	57
Total	85	29	114

$X_1^2 = 0.42$ (NS)

Alveolar Internal Surface Area

	L	U	Total
NO_2	41	16	57
air	44	13	57
Total	85	29	114

$X_1^2 = 0.42$ (NS)

Linear Intercepts of Alveolar Walls

	L	U	Total
NO_2	40	17	57
air	45	12	57
Total	85	29	114

$X_1^2 = 1.16$ (NS)

Alveolar Wall Area ÷ Number of Type II Cells (>10 μm)

	L	U	Total
NO_2	48	9	57
air	37	20	57
Total	85	29	114

$X_1^2 = 5.60$ ($0.01 < p < 0.025$)[c]

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[a] Each animal is ranked into the upper quartile (U) or the remainder (L). A significant chi-square value indicates an association between membership in a particular group and membership in the upper quartile. NS = not significant ($p > 0.1$).

[b] Same for Type II cells of >10 μm and >12 μm.

[c] For Type II cells of >8 μm: $0.05 < p < 0.1$.

Protein Content of Lung Tissue Following Exposure to Nitrogen Dioxide

In other studies, we used a molecular probe, horseradish peroxidase (HRP), to measure the protein content of lung tissue from NO_2-exposed mice. In brief, intermittent exposures of Swiss-Webster adult male mice to NO_2 at levels as low as 0.4 ppm for periods of 3 and 6 weeks resulted in a greater content of HRP in the lungs of exposed animals as quantitated by polyacrylamide gel electrophoresis (Sherwin et al. 1979b). In three independent experiments, with two test periods per experiment, exposed animals showed greater mean HRP values for five of six periods (upper quartile analysis). Using a two-factor analysis of variance alone, statistically significant differences were found in three of six periods. Ultrastructural studies of animals injected with the HRP probe showed HRP to have free access to the basal lamina of the lungs of both control and exposed animals; studies of the electron micrographs (incomplete at this writing) have shown no differences in distribution. Pinocytotic vesicles of both endothelium and epithelium appear to play major roles in the transport of HRP from the capillary to the alveolar lumen as part of the bidirectional protein transport mechanism.

DISCUSSION

While the findings reported here are preliminary, they are in line with earlier reports showing both Type II cell hyperplasia and protein leakage at higher levels of NO_2 exposure. Other studies are in progress to confirm and elaborate upon these preliminary results (see Addendum, below). The question of reversibility is of special concern. It should be noted that Type II cell hyperplasia and protein leakage are very early findings and common denominators in diverse kinds of human lung disease.

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ADDENDUM

At the time of review of the draft Proceedings, data from another study had again shown hyperplasia and hypertrophy of Type II cells. In this study, mice were exposed to 0.3 ppm NO_2 for 6 h/d, 5 d/week, for 6 weeks, with tests at 4 and 10 weeks post-exposure. Also, comparisons of the three test periods showed impaired mitochondrial growth (size). The mitochondrial and Type II cell alterations persisted to the 10-week post-exposure period.

37. PULMONARY AND PSYCHOPHYSIOLOGICAL EFFECTS OF OZONE

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INTRODUCTION

This report summarizes some preliminary results of human clinical exposures to ozone (O_3) performed at the Institute of Environmental Stress at the University of California - Santa Barbara. Results of some of the individual studies have been published elsewhere (Folinsbee et al. 1978, 1980; Gliner et al. 1980; Horvath et al. 1979).

A special focus of our work is the pulmonary functional response to O_3 in combination with exercise. Another focus is the habituation response to O_3 (and individual differences in that response). Finally, recent studies examine some effects of O_3 on the central nervous system.

PULMONARY EFFECTS OF OZONE IN COMBINATION WITH EXERCISE

Exercise at a fixed level of ventilation had dramatic effects on pulmonary function following O_3 exposure. At 0.5 ppm O_3, we observed marked pulmonary function decrements immediately following 15 min of exercise (regardless of the time at which the exercise occurred). Variations in the amount of decrement reflected individual sensitivities to O_3. Prolongation of the exercise to 30 or 60 min resulted in higher decrements. In addition to exercise, environmental temperature was implicated in the degree of pulmonary function decrement.

Other studies examined the capacity for exercise following O_3 exposure. We observed a 10% decrement in maximal exercise capacity following a 2-h exposure to 0.75 ppm O_3 during which subjects exercised intermittently. A slight reduction in the O_3 concentration combined with omission of the intermittent exercise resulted in no decrement, however.

In all such studies, it is very important to directly measure and control the subject's level of ventilation. Attempting to infer ventilation from heart rate, etc. (as in some studies) is not adequate. We performed various O_3 exposures (0, 0.1, 0.3, and 0.5 ppm) at various subject ventilation levels (10, 30, 50, and 70 liters/min) and confirmed that forced vital capacity (FVC), forced expiratory volume (FEV_1), and other parameters decrease markedly and consistently with the level of ventilation, and more so with the level of inspired O_3.

Knowing the subject's ventilation level during periods of exercise and rest allowed us to calculate the effective dose in each experimental trial. Knowing the effective dose permitted us, in turn, to construct straightforward equations that predict pulmonary function decrement as a function of three variables: ventilation, concentration, and exposure. We are confident of the validity of these equations: all are significant at $p < 0.01$.

PULMONARY HABITUATION TO OZONE IN COMBINATION WITH EXERCISE

Habituation exposures were performed under a protocol similar to that described by Haak (Chapter 31 of this volume). However, our subjects practiced for 2 weeks prior to exposure, allowing us to obtain base-line pulmonary function measures. Exposures to filtered air and O_3 (0.2, 0.35, and 0.5 ppm) extended for 3 or 5 d. Relative humidity was maintained at 45%, to simulate the environment of a hot summer day in Los Angeles.

Evaluations of several pulmonary function parameters (including FEV_1 and FVC) revealed a general pattern: a maximum decrement on the first or second day of exposure followed by a return to near-normal on the third or fourth day. Despite this general pattern, however, individual subjects displayed considerable variability in habituation. For example, one subject showed a dramatic fall in FEV_1 on the first day of exposure but had fully recovered by the second day; no further change was seen. A less sensitive subject showed a slight change in FEV_1 on the first day of exposure but no decrement thereafter.

At present, we are following the highly sensitive subjects by having them return to the laboratory at various time intervals. Our goals are to determine the length of time for which habituation persists and to identify the mechanism by which habituation occurs.

PSYCHOPHYSIOLOGICAL STUDIES

Studies of subjective symptoms in O_3-exposed subjects showed a marked correlation between the number of symptoms and the environmental temperature.

In studies of vigilance task performance, O_3-exposed subjects showing significant pulmonary function decrement in the first hour of exposure showed a gradual decline in the ability to detect signals during the second hour of exposure. The most recent studies suggest a correlation between vigilance performance decrement and O_3 concentration.

Preliminary studies of O_3 effects on the electroencephalogram (EEG) suggest a decrement in the usual 8-10 Hz range.

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WORKSHOP COMMENTARY

R. K. Wolff: Regarding the temperature effects, does the subject's ventilation go up at a higher temperature? Why do you think temperature has that effect?

S. M. Horvath: Body temperature goes up. At a higher temperature ventilation increases, so we lower the work rate in order to maintain the ventilation constant. In our view, [illegible] look at the effects of O_3 on the pulmonary functions of man requires at least a constant ventilation. If you don't have a constant ventilation, all you're looking at is variability. As a consequence, there is less variability in our obtained data simply because we spend so much time looking at and measuring the ventilation. We know the maximum work capacity of each subject and can predict within 5% what level of work will maintain a certain ventilation. We continually monitor the ventilation and lower or raise the work rate to maintain the ventilation.

Question: Does your "effective dose" refer to absorbed O_3 or inspired O_3?

S. M. Horvath: Inspired O_3. We were originally interested in measuring how much O_3 is really taken up. The exposure level does not tell you what amount actually gets into the lungs. If the Air Resources Board can provide the funding, we will obtain that information. In the meantime, we still don't know how much actual O_3 is taken up by the individual. We do know how much is presented, and the prediction equations are based entirely on that.

38. EPIDEMIOLOGIC STUDIES OF OXIDANT HEALTH EFFECTS IN THE LOS ANGELES AREA

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INTRODUCTION

Since 1972, the University of California - Los Angeles (UCLA) and the Los Angeles County Lung Association have collaborated on studies of the relationship between chronic exposure to various types and levels of air pollution and lung function test performance. The primary objective of these studies is to determine if changes in lung function test performance over time can be correlated to residence in areas exposed to high and moderate levels of photochemical oxidants. No previously reported studies have attempted a similar correlation in a large, free-living population; thus, our project may be definitive in establishing the magnitude of such correlation (if any). Coinvestigators for the project include this author as well as Dr. Stanley Rokaw, Dr. Frank Massey, Dr. Donald Tashkin, and Mrs. Anne Colson.

To date, we have completed base-line testing in four areas and retesting in one of those four areas. This report presents some preliminary results of a cross-sectional study completed for the years 1972-1977. There are many problems associated with the cross-sectional study design; these can be resolved, in part, by completing longitudinal follow-up of the cohorts already established.

STUDY SITES

Four study sites were selected to provide a range of pollution exposures. Glendora, located in the East San Gabriel Valley, is exposed to high levels of photochemical oxidants. Burbank, in the East San Fernando Valley, is exposed to moderate levels of photochemical oxidants. Lancaster, located two mountain ranges north of downtown Los Angeles, is exposed to low levels of all ambient air pollutants. The Long Beach site is exposed to low levels of photochemical oxidants but relatively high levels of sulfur dioxide, particulates, and (presumably) hydrocarbons.

These four sites were also selected for contiguity to monitoring stations of the Southern California Air Quality Management District, which can provide continuous measurements of levels of selected air pollutants. Monitoring stations are located immediately adjacent to or within the four study sites with the exception of Glendora, where the monitoring station at Azusa (~2-3 km upwind of the study site) is used to estimate exposures.

The monitoring data we compared consisted of average annual means of daily maximum hourly average concentrations of selected air pollutants over the six study years. The Azusa station had the highest levels of photochemical oxidants. Lancaster showed oxidant levels that were higher than originally expected but lower than the levels in Burbank and Azusa. Long Beach had the lowest levels of oxidants but the highest levels of sulfur dioxide. Nitrogen dioxide levels were highest for Long Beach and Burbank. Particulates were high in Azusa. Particulates were not measured in Long Beach, but isopleths indicate that Long Beach had the highest particulate levels. Hydrocarbon measurements were a problem because they were not made in Long Beach until 1977. We had hoped that one result of the planned joint ARB/EPA studies would be better measurements of hydrocarbons and particulates.

PULMONARY FUNCTION TESTING

Subject Recruitment

At the beginning of each test period, articles concerning the research program appeared in the local newspapers, and letters of introduction were sent to residents of the target areas sequentially so as to permit lung function testing within one week of notification. Telephone contacts and construction of household rosters were done by individuals recruited from the communities in which the testing was being carried out.

The percentage of households enumerated ranged from 84% in Lancaster to 98% in Glendora. The proportion of residents completing lung function testing ranged from 70% in Burbank to 79% in Lancaster; the actual number completing lung function testing ranged from 3403 in Glendora to 4509 in Lancaster. An additional 1 to 8% of residents of the four areas completed a respiratory questionnaire only. The demographic characteristics of the nonrespondents were similar among the four study areas.

The prevalence of residents with a history of asthma, bronchitis, or emphysema ranged from 10 to 13% in the four communities and was highest in Lancaster, the study area exposed to the lowest levels of pollutants.

Lung function testing was completed only on residents who were seven years of age and older. We felt that children younger than seven years of age would not be able to follow the procedures. Also, it was necessary that each subject be able to walk up the steps to the mobile lung research laboratory. Individuals who were so infirm as to not be able to walk up to the laboratory could not be tested, although we were very willing to assist them if possible.

Testing Protocol

Prior to pulmonary function testing, subjects underwent an interview schedule administered in a separate trailer. Lung function tests were administered in a mobile lung research laboratory. These tests included: forced expiratory spirometry with computer recording of the entire flow volume curve; the single breath nitrogen test, including the change in level of nitrogen between 750 and 1250 cm^3 of expired air as well as the closing volume fraction; and body plethysmography, including determination of thoracic gas volume, functional residual capacity, and specific airway resistance. (We were quite surprised at the very low refusal rate for plethysmography: <1%.) Each spirometric flow volume curve was recorded directly onto tape so that we could select best breath, best two breaths, etc. using computer algorithms. The results of the other two tests were recorded by hand.

Quality Assurance

Several strategies were used to determine the reliability of the lung function testing: (1) every tenth participant was immediately retested; (2) 100 residents of each area were retested three times over the course of a year; (3) a 3% probability sample of individuals completing lung function testing was reexamined using more intensive techniques at the UCLA pulmonary function laboratories. In addition, instruments were calibrated and recalibrated several times during each test day. Reliability for the spirometric indices was excellent; for the plethysmographic indices--fair; and for the single breath nitrogen indices--poor.

PRELIMINARY RESULTS

We have completed a preliminary analysis of the following parameters: cough and sputum, any symptom, forced expiratory volume (FEV_1), peak flow, and closing volume fraction. In order to minimize the number of variables between study groups, the results presented here are limited to white residents who did not have Spanish surnames and who had never changed residence or occupation because of a respiratory problem. Further, these results are only for individuals who were either current smokers or who had never smoked, and includes only individuals who were 25 to 59 years of age. Analyses of results for younger subjects, older subjects, and former smokers are ongoing.

Among residents who had never smoked, the worst test values were most frequent in Glendora, the area exposed to the highest levels of photochemical oxidants. The frequency of worst test values among participants from Burbank (the area exposed to moderate levels of oxidants) was low and similar to the frequency among residents of Lancaster (the area exposed to low levels of all pollutants). The second highest frequency of worst test values occurred among residents of Long Beach (the area exposed to relatively high levels of sulfur dioxide, particulates, and possibly hydrocarbons).

Among current smokers, the distribution of worst test values was similar to the distribution among never-smokers. Two of the tests that presumably measure predominantly small airway function ($\Delta N_{2_{750-1250}}$ and the maximum flow at low lung volumes) correlated with smoking but not with residence. Differences in results (any test) between the best and the worst study areas were greater than differences (same test) between never-smokers and current smokers.

The results of the spirometric tests in the Burbank participants were closer to those in the Lancaster participants than in the Glendora participants, suggesting the possibility of a nonlinear dose-response curve or a threshold value for oxidants lying somewhere between the levels occurring in Burbank and the levels occurring in Glendora. Another possible explanation is that more affected residents of the Burbank study area had moved away before the program started. This possibility will be resolved by longitudinal follow-up of the established cohorts.

In summary, the highest frequency of worst test values occurred among participants in the area exposed to the highest levels of photochemical oxidants, and the lowest frequency of worst test values occurred among participants in the area exposed to the lowest levels of all ambient air pollutants.

DISCUSSION

These results should not be viewed as conclusive evidence of a relationship between chronic exposure to photochemical oxidants and impairment of lung function. Although most of the biases we have been able to identify would tend to decrease observed differences in comparison to true differences between the communities, cross-sectional studies cannot account for all of the variables which may confound these comparisons. Thus, it is essential that we pursue the original objective of these studies: to observe whether changes in lung function test performance are commensurate with the levels of the various pollutants measured concurrently over the five-year test interval.

WORKSHOP COMMENTARY

Comment: Case-study epidemiology is always a tough thing, and you left yourself a good caveat. Still, with regard to oxidants, should you be measuring people where they live or where they work? Shouldn't you be looking at people who work outdoors--telephone linemen, ditch diggers, and so on--to get some idea of what exposure to those ambient concentrations means? Spizer and Ferris and others have found very large differences between ambient levels of photochemical oxidants and levels of photochemical oxidants indoors where, unfortunately, most of us spend our busy days.

And how about the correlation with income that is seen in the epidemiology of cancer in large cities (high income, low cancer)? Does this reflect the fact that people who don't have much money always live down by the bayou, down in the valley, next to the railroad tracks, close to the factory? It's never clear to me which is cause and which is effect, but this is something for you to consider here. I suppose that you have considered it.

R. Detels: Yes, we have. First, with regard to socioeconomic status, I'm afraid I didn't make it clear that we matched these communities on mean income, on cost of housing, and on racial distribution. That's why they are predominantly white. If we had taken any other kind of a mix it would have been impossible to match them and have different air pollution exposures.

With regard to place of residence vs. place of employment, we took down histories of where they live and where they work, how much time is spent in commuting, etc., and we'll be looking at that. However, Lancaster is ~60-70 mi from downtown Los Angeles. Therefore, the majority of residents do not make that commute. The same is not true for the people from Long Beach, Burbank, and Glendora, which are the three polluted areas. People from Glendora only have "one way to go," and that's to reduce their pollutant exposure by commuting. Therefore, it is our impression that the biases that would be introduced by commuting to an area of different pollutant exposure would tend to diminish the observed differences from the true differences. However, we will be looking at subgroups on the basis of commuting patterns.

We also take occupation into consideration. We know that the distribution of occupations is similar in these four communities.

Question: What do ambient values have to do with the inside of a Lockheed or Douglas plant?

R. Detels: I can't answer that. But I think that if one sees differences in breathing parameters between communities, then one has to hypothesize differences--occupational or other exposures--if one is unwilling to accept residence as the important variable. We found no differences in occupations that could account for these differences.

We haven't looked at the difference between indoor and outdoor exposures. Frankly, that's difficult in a residential type of study. The object of this study is to look at whether breathing ability is associated with air pollution levels in the area of residence. We realize that this objective has some shortcomings; if we see no difference then we can't say much. However, if we see a difference, the argument against a residential effect is a little harder to make.

Question: Would you explain the kinds of exposure data that were available to you?

R. Detels: Data were available for a seven-year period from continuously operating stations of the Southern California Air Quality Management District.

Question: And you had access to the exposure data from that time up to the current time? At what frequency?

R. Detels: The Southern California Air Quality Management District's monitoring is continuous. The Air Resources Board and the Southern California Air Quality Management District have kindly provided us with their measurement tapes so that we can select any parameter we want. We picked the mean of the daily maximum. However, one can select other parameters. Dr. Yuji Horie of the Technical Services Division has looked at exposure in terms of the number of days over the air quality standards for these areas. That is another way of trying to document pollution differences. Using that technique, the differences are even more dramatic. We have data dating back to the early 1960's.

Question: Have you done any analysis in terms of length of residence?

R. Detels: We have that information. We deliberately picked communities having a high proportion of individuals who had lived there five years previously. We did not divide it into individual years of exposure in that community.

When you stop to think about it, if somebody has lived in Burbank for 5 years but spent the previous 20 years in downtown New York City, how does he compare to somebody who has spent 5 years in Burbank and the previous 20 years in Apple Valley, California? I don't know. We have the information, but we don't know how to get at that.

Question: For your follow-up, do you plan to repeat all the same measurements? We have a concern, for example, in terms of the single breath nitrogen test. You indicated that there was very poor reliability.

R. Detels: That's correct. First of all, it's a gratuitous measurement. The instrumentation is on the test unit. To take it requires an extra 3-4 min of the participant's time and little else. Second of all, we did see an association with closing volume in the four areas and we did see a correlation with cigarette smoking. If we're going to look at these tests to see whether they're reasonable predictors (one of the subobjectives of this study), it's important to see that the test itself is consistent in an individual over a five-year course. If we find somebody with a low closing volume (which we think may be an early sign of respiratory disease) in 1973 and then we find him with a decreased FEV_1 but a good closing volume five years later, I would question whether the closing volume is a very good predictor. I would think that the closing volume measurement must at least be consistent with what we saw in 1973.

If we had to go out and buy all of that instrumentation from scratch in order to do the retesting, there would be a very reasonable question raised. But since it's gratuitous and we can get that consistency estimate, I think we would be somewhat remiss not to do it.

Comment: That's my point. It may be consistent but it may not be reliable.

R. Detels: In my opinion, in order to be consistent it must be reliable. It may not be sensitive, it may not be specific, but it must be reliable.

F. J. Miller: What percent of response did you get in your follow-up?

R. Detels: This is one of our urgent concerns. In the follow-up for Burbank, we can account for ~84% of the cohorts. For ~16% we have demographic information and base-line test results but no retest information. Our problem is that people moved out of Burbank. Unfortunately, we can't bring the mobile laboratory to these people because they are scattered over a wide area. Therefore, we have to ask them to drive several hours, if they live in Southern California, to be retested. Retesting of individuals who have moved further away is completely unfeasible. Most of our refusals were, in fact, from people who had moved.

To date, we have completed ~75% of the testing in Lancaster. At this point, it appears that the response rate will be better than in Burbank.

The response rate in the remaining two areas will be better because we initiated the annual follow-up about a year and a half after the original Lancaster cohort was done. Unfortunately, for two years we had no follow-up in Burbank; this may be part of our problem. I want to emphasize that, the longer we delay, the more people will move out and the lower the response rate will be.

F. J. Miller: Are they moving from the area or are they just not responding, to avoid the bother of testing? Also, you can minimize your differences by the fact that they choose to move out of the polluted area.

R. Detels: We send a letter to each individual who has moved away. If there is no response, we send a second letter. Finally, we telephone the individual. One of the major questions we ask is: "Why did you move?" So, if they moved for respiratory reasons, we have that information. In addition, we will compare the lung function test parameters at base line for those who moved out vs. those who remained. We want to determine if there are differences between these two groups. We did that using FEV_1; there were no large differences. We are also looking at the demographic characteristics, since we have complete questionnaires on all subjects.

Thus, we have better documentation of the characteristics of our retest nonrespondents than most studies do. We know a lot about their respiratory physiology at base line, and we know a lot about their demographic characteristics.

Comment: Are there sufficient numbers of people who move from an area because of respiratory problems to make it worthwhile to do a correlation of their response? In other words, let's assume that certain people can adapt to photochemicals in the area and others cannot. Those who cannot adapt, move; they would be a subgroup that might be very interesting to study.

R. Detels: That's true. As long as they move within the general Southern California area, we have that potential. We could either bring the laboratory to them or bring them to the laboratory.

W. Frietsch: Could you briefly describe this study's origin? What were the major objectives?

R. Detels: There were two reasons the study came about. First, I moved into the Los Angeles area about 1971 and decided that if I was going to breathe it, I might as well study it. About that time, the National Heart Lung Blood Institute issued a request for proposal for a population study of areas exposed to air pollution. We responded with a proposal to look for the changes in lung function parameters in groups of people exposed to different levels of photochemical oxidants and other pollutants over a five-year period. That remains the major objective. Now, we have a whole host of subobjectives. But I want to emphasize that it was not designed as a cross-sectional study. We were well aware of the pitfalls of the cross-sectional study. Still, that remains the best way to form a population-based cohort.

F. J. Miller: From all the caveats you have mentioned (in terms of analyses that could be done, etc.), my general reaction is that there are a couple of years of analyses to perform before you can really consider an expanded study or something on the order of "goals." It appears that you have a tremendous amount of data that still need to be interpreted and put in perspective for an effective study.

R. Detels: You're absolutely right. Given the best of all possible worlds, I would have tested all these communities at the same time. Then I would have "put them in the icebox" and said, "Don't anybody move; it's going to take me two years to go through the analyses I'd like to do." Such analyses would not be associated with the ultimate objective, and none would overcome the problems of a cross-sectional study. But such analyses would involve very interesting cross-sectional studies: comparisons of different areas, comparisons of different tests, comparisons of different age groups, comparisons of different analytical strategies, and so on. The problem is that subjects continue to move. The longer we delay the fewer people will be available for retesting.

The second problem is that two measurements taken over five years and used to estimate mean annual change cannot be compared to a mean estimated from two measurements taken over seven years. The actual rate of change in lung function parameters may not be constant over time. Such a comparison assumes that the rate of change is linear. I'm almost sure that it is in fact not linear. For instance, one of the groups in which we're most interested are those who are progressing from young adulthood to adulthood. Their changes in lung function are certainly not linear. And I'm sure there are other groups in which the changes are not linear.

Limited funding has forced us to make compromises. When cuts were necessary, those cuts were made in analysis. You cannot cut the collection of field data. Without collection of data there is no study.

We have an opportunity to do all the analyses, but if we do only the analyses we'll lose the cohort. If we lose the cohort, we're stuck with another cross-sectional study and all of its attendant problems. So, although I would prefer to stop and do two or three years of thorough analyses, I don't think we have that option. We did not have that option in the past because we had to focus on the collection of quality data in which we could have confidence. Had we diverted the funding from collection of data to analyses, I'm not sure that we would have obtained anything worthwhile to analyze.

39. PROPOSED SCIENTIFIC PROGRAM OF THE COOPERATIVE STUDY OF OXIDANT HEALTH EFFECTS IN THE LOS ANGELES AREA

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INTRODUCTION

This report describes a program to further our knowledge of oxidant effects on human health in the Los Angeles area. The primary aim is to reduce uncertainty about health implications of ambient air quality standards. Funded jointly by the California Air Resources Board (ARB) and EPA, this program consists of an integrated set of human and animal studies to be conducted cooperatively over the next five years. Table 39-1 summarizes these various studies. Most of the initial work will use established methods, but innovative approaches will be considered in the program's evolution.

EPIDEMIOLOGIC STUDIES

The key question of how variously polluted atmospheres relate to measured health effects in populations is being addressed in a large population study

TABLE 39-1. COMPONENT INVESTIGATIONS

Atmosphere	Group Studied	End Point(s)	Investigator, Institution
ambient	census tracts (4) emergency room walk-in's asthmatics	lung function, questionnaire chest complaints medication, lung function	R. Detels UCLA
0.3 ppm O_3	human sample	lung function	J. Hackney Rancho Los Amigo Hospital
pollutant mixtures (oxidant + sulfurous)	exercising rats exercising dogs	clearance + morphometry lung function + morphometry	T. Crocker UCI
0.5 ppm O_3 2.0 ppm O_3	young dogs	terminal lung function + morphometry	R. Phelen UCI
0.2–1.2 ppm O_3	young rats young monkeys	extensive morphometry (ongoing function)	W. Tyler UCD
0.2–0.8 ppm O_3	monkeys	macrophage motility (ongoing structure)	L. Schwartz UCD

by R. Detels and associates at the University of California - Los Angeles (UCLA) (see Chapter 38 of this volume). In this study, one full round of pulmonary function testing and questionnaires has been completed in four census tracts (4000-6000 population each, aged 7 and up) located in variously polluted regions in the Los Angeles area. Follow-up studies of individuals originally tested have been completed (84% retention rate) in Burbank (high oxidant, medium sulfur dioxide (SO_2)) and nearly completed in Lancaster (low oxidant, lowest SO_2). Follow-up studies will begin in Long Beach (lowest oxidant, highest SO_2) in spring 1980 and in Glendora (highest oxidant, medium SO_2) in 1981.

The initial part of this study, a cross section of the four census tracts, has already yielded some useful results. Pulmonary function test results were more below normal in the more "smoggy" areas, but the relationship between test results and pollutants was not simple. Because of the potential for different population conditions in different areas, such cross-sectional studies are thought to be of limited validity in establishing the relationship between mixed pollutants and health effects. The serial or follow-up study of pulmonary function decrement in given individuals over time will permit a more direct assessment of pollutant effects on populations in the four tracts. Preliminary results of the follow-up study show greater pulmonary function decrement in Burbank than in Lancaster, an area of substantially less air pollution of every measured species.

The UCLA group plans two additional smaller-scale epidemiologic studies. Tentatively, the first will involve a reporting network for chest complaints by emergency room "walk-in's." The other will be a comparative asthma panel study in districts that have distinctly different oxidant levels but are matched in other ways. The use of medication dispensers that can provide assured counts and the use of quantitative self-testing will be explored.

THE INTEGRATED PROGRAM

In developing an integrated program that starts with these epidemiologic studies, we sought projects that might answer questions not addressed in purely epidemiologic studies, and we sought to add new exposure information to sharpen the epidemiologic studies themselves. One limitation of epidemiologic studies is the lack of confidence that one can place on the effect of a particular level of a particular pollutant, while a strength of the Detels cohort study is the determination of actual rate of lung function loss. An exposure chamber study, on the other hand, can provide a clear response to a particular level of pollutant, but the long-term significance of that immediate response is open to question. Our integrated program proposes to directly link these two types of studies by drawing samples for an exposure study from Detels' well characterized epidemiologic cohorts subsequent to follow-up tests of pulmonary function.

As a further aid in interpreting the epidemiologic studies, four animal studies are planned. Using rats and dogs under carefully controlled exposure conditions, the first study will examine the lung damage and impairment attributable to two important environmental influences: exercise and pollutant mixtures (both key factors in Los Angeles). The second and third animal studies will address questions for which the Detels data are not yet analyzed: namely, effects on the very young. Experiments on dogs, rats, and monkeys will examine the extent of lung damage due to ozone (O_3) exposure during the development of the lung, with special reference to the stage of alveoli formation. Lung damage will be assessed morphometrically in all three of the animal studies--a difficult approach in humans, although a comparative post-mortem study on human adults is a future possibility. The fourth animal study will explore a mechanism that is considered to be a likely key to the accelerated loss of lung function that may occur in polluted regions of Los Angeles as well as to the damage observed in terminal bronchioles of animals exposed to as little as 0.2 ppm O_3. The hypothesis is that low levels of O_3 impair alveolar macrophage motility and that stagnant macrophages release enzymes which attack the unciliated bronchiolar epithelium. The approach of the study is to lavage (wash) and then measure the motility of macrophages from certain lung segments of O_3-exposed monkeys. The lavage technique carries future potential for assessment of the human lung condition relative to pollution exposure.

IMPROVEMENT OF POPULATION EXPOSURE ESTIMATES

Some program resources have been set aside for exploring ways to improve exposure estimates in the Detels study. This work will be conducted early in the overall program.

The first aspect of this project is to ensure that the basic data from the monitoring stations are as comprehensive and complete as possible. A few standard pollutants were not actually measured during the study, though all standard pollutants are now scheduled for routine monitoring in the two remaining tracts. In addition, there is an important opportunity to measure fine particles in all four stations near the tracts.

This effort involves two further aspects. One is the question of how the actual exposures of individual subjects might have differed from the values reported at the monitoring stations. Measurements and/or modeling of local sources and indoor variations may be helpful in answering this common epidemiologic question. The other question is of how individuals may have been temporarily affected by pollutants at the time of the pulmonary function measurements.

HUMAN LABORATORY EXPOSURES

The primary exposure of subjects from epidemiologic cohorts will be performed by J. D. Hackney and associates at Rancho Los Amigos Hospital in

Downey, California. Fifty subjects from the Burbank cohort will be exposed to 0.3 ppm O_3 for ~2 h. The preliminary design calls for half of these subjects to be drawn from the group that experienced the most pulmonary function loss and for the other half to be drawn from the group that experienced the least loss. Testing of the immediate response to O_3 will determine the significance of any correlation with loss of function over five years.

Should a significant positive correlation between long-term pulmonary function loss and short-term O_3 sensitivity be found, two contrasting explanations could be offered. One explanation would be that lungs which are a priori most responsive to O_3 insult in the short term are also most susceptible to long-term function loss. The other would be that existing pulmonary function loss predisposes the lung to short-term O_3 sensitivity. Either explanation would carry importance in standard-setting, and future studies (e.g., similar studies of groups with less O_3 exposure or longitudinal studies of individuals with high and low short-term O_3 sensitivity) might be able to distinguish between them. Related studies by Hackney and others support the possibility of detecting relatively small but significant O_3 responses and intergroup differences in response. However, the likelihood of successfully detecting differences between groups showing different long-term pulmonary function loss is difficult to predict. Even if the attempt to distinguish between groups is not successful, considerable additional information on responses to "worst case ambient" O_3 levels will have been obtained on a group of Southern California residents who, though still self-selected, bear a well defined relationship to a typical middle-income community population.

Because of the phenomenon of desensitization or adaptation to O_3 exposure, it seems likely that the clearest test of immediate O_3 responsiveness of these subjects would be performed relative to an external atmosphere that is as free of pollutant as possible. Thus, special precautions will be taken to reduce effects of environmental background: we intend to work with our subjects during seasons of low smog and to estimate their environmental pollutant exposures. In subsequent years, it may be possible to arrange for a portable exposure chamber for the two most distant census tracts. Ultimately, it may still be worth transporting subjects to a cleaner environment (e.g., Santa Barbara) for several days of tests. General animal studies of the phenomenon of desensitization and of the correlation between immediate O_3 responsiveness and rate of pulmonary function loss during chronic exposure would provide useful correlates to these human studies.

Subjects who participate in the human laboratory exposures could be measured in various new ways with no additional inconvenience to them. A proportion of program resources support pilot studies of innovative ideas that appear likely to yield results. With A. Richters at the University of Southern California we are considering exploratory studies on the blood samples that will (in any case) be obtained. Leukocyte population counts and leukocyte function tests of both T cells and B cells might reveal differences between groups. In addition, with J. F. Mead at UCLA we are considering exploratory studies of exhalate to measure pentane concentration as a marker of peroxidation in the lung lining.

ANIMAL LABORATORY STUDIES

A study to examine the effect of exercise on dogs and rats exposed to pollutant mixtures for short periods is planned by T. Crocker and associates at the University of California - Irvine (UCI). That laboratory has developed exposure chambers which permit careful control of the aging of atmospheric pollutant mixtures. In the initial tests, the atmospheres will consist of:

1. Toxic hydrocarbons produced by photochemical reactions, including formaldehyde, acrolein, or peroxyacetyl nitrate alone at 0.1-2 ppm
2. O_3 alone at 0.2-0.6 ppm
3. Particulate ammonium sulfate at 1 mg/m^3 plus SO_2 at 0.5-5 ppm
4. Combinations of (1.) and (2.) and of (2.) and (3.)

The hydrocarbons and SO_2 are water soluble; hence, combined hydrocarbon-particulate or SO_2-particulate atmospheres will be studied at low and high humidities. Beagle dogs will be exercised on a refrigerated treadmill; pulmonary function will be monitored by face mask, esophageal balloon, expired gas, and carotid blood gases. Rats will also be exercised; the post-exposure ability to clear inhaled particles from the lung will be measured. Detailed lung morphometric measurements will include characterization of lesions and of cell replication.

Studies of lung development are planned initially for O_3 exposures only. R. Phelan at UCI will expose beagle puppies to 2.0, 0.5, and 0 ppm O_3 for 4 h/d for 5 d at an age of active development, based on a protocol successfully developed for 1.0 ppm. The facility for housing the dogs maintains an exceptionally pure environment. After development is complete, the lungs will be subjected to pulmonary function tests and detailed morphometry using special methods for preparing the microscopic sections. Mean linear intercept will be measured. W. Tyler at the University of California - Davis (UCD) plans to augment and continue rat and monkey studies that are in progress. The rat studies are both episodic (0.8 ppm O_3 for 3 d during various stages of development) and chronic (1.2, 0.8, and 0.2 ppm). Measurements by the most advanced morphometric methods will include internal surface area, capillary volume and surface area, and mean thickness of the air/blood barrier. The monkey studies are chronic, involve an exposure level of 0.8 ppm O_3, and start at 6 mo of age; measures of pulmonary function and extensive morphometry will be completed (as in the rats).

L. Schwartz of UCD will perform lavage of alveolar macrophages on healthy monkeys exposed to 0.2-0.8 ppm O_3. The macrophages produced by lavage will be examined in a number of ways; the primary approach will be to measure motility. The response to lung lining material and chemotactants will receive attention. Data on macrophage structural alteration (obtained in ongoing studies) will be compared to the observed functional changes.

OVERALL ASPECTS

An unusual feature of this program is the inclusion of a statistical working group both as an aid to investigators and as a management mechanism. Nine distinguished statisticians from Stanford University, the University of California - Berkeley, and UCLA have agreed to join a Design and Evaluation Committee coordinated by L. Breiman, an independent statistical consultant based in Santa Monica. Operating under very specific guidelines, the Committee will be responsible for the following functions:

1. To critically review all experimental design for statistical integrity and power, including design modification at the proposal stage and as necessary thereafter to ensure statistical integrity and power;
2. To critically review the statistical analysis of all experimental data;
3. To actively assist in the analysis of all data;
4. To develop a continuing evaluation of the statistical implications of all results bearing on oxidant standard-setting.

In addition, the ARB program management staff will be responsible not only for coordinating the investigations but also for synthesizing the results. Some of this synthesis--especially that involving extrapolation of animal results to man--may employ mathematical modeling approaches as well as biological concepts. The most useful overall contribution of the synthesis may be to obtain, in connection with inputs from the statisticians, a clearer definition of the degree of confidence that can be attached to statements of oxidant effects at ambient levels.

WORKSHOP COMMENTARY

Question: Could you outline the approximate resources that would go to each of the studies? Also, how did you arrive at that emphasis in terms of meeting study objectives?

S. V. Dawson: To answer the last part of your question, we looked at the merits of each study and the relevance of each in combination with the others. To answer the first part, with respect to [Table 39-1], the Schwartz, Tyler, and Phelan studies are each funded in the range of $70,000 to $90,000 a year; the Crocker study is funded at $200,000; the Hackney study is funded at $150,000; and the Detels study is funded at $530,000.

Comment: In my understanding, the original question to be answered by the study concerned the health effects that occur in Los Angeles, since in Los Angeles the standards (particularly for O_3) are fairly routinely exceeded. After all the negotiations, is that still the major objective of the study? Does the design really focus in on that question?

S. V. Dawson: I would certainly claim that we're looking for health effects caused particularly by levels of O_3 and oxidant in Los Angeles. All of these projects were screened for relevance to that question. It's true that, in terms of pollutant, four of the studies address only O_3. In another exposure experiment, O_3 is added on to other things (the sulfurous compounds and the rest of the total oxidants). We have had trouble, perhaps, distinguishing between O_3 effects and oxidant effects. This is at least a step in that direction.

Question: Were the studies assembled in sequential order? Did you really look at the major objective and design a study to achieve that, or did you more or less pick up pieces of different projects and assemble them?

S. V. Dawson: From the statements of interest we received, we assembled those pieces that were most relevant to our objective and that were of sufficient scientific merit. Several of the submissions were substantially modified; we went pretty far in that direction to actually shape the thing but, as you know, investigators have certain interests and you can only move them a bit. To a certain extent, one has to take what one can get in the real world.

Question: What documentation for your presentation exists now?

S. V. Dawson: The basic scientific program is outlined just as presented here.

J. A. Graham: I have three or four general questions. First, I don't understand the rationale for doing two studies on the young until results are available from at least one of them. Obviously, it's nice to work with a number of animal species, but I don't see the rationale for doing all of this work with O_3 and no work with NO_2, for example. I don't see the rationale for duplication without prior data.

S. V. Dawson: Your point is well taken, but the investigators do have some interesting results. Phelan has some very interesting results with that 4% shift. Tyler has some very significant results on the alveolar-volume-to-body-weight ratio, and his proposal is to do detailed morphometry to look at that. So, as far as rats and dogs go, the investigators are producing some very interesting results on O_3. But the Committee is also very intrigued with the monkey study. The monkey lung is more like the human lung and, if the hypotheses about damage are right, the monkey should be very important for looking beyond the rat and dog.

J. A. Graham: I guess that should be included in the more detailed proposal, for the purpose of comparing results with those from extensive studies in this area by Dr. Freeman.

My second comment concerns the O_3 concentrations. I don't see how studies at 2 ppm or even 1.2 ppm are going to be highly relatable to the rest of the studies: those are very high concentrations compared to ambient levels.

S. V. Dawson: First of all, Phelan is the only one that has suggested 2 ppm. I argued that you really only need a couple of pairs of animals at 2 ppm to get sufficient power in your test. This would indicate the direction in which the line is going, thereby giving you a better calibration for the very difficult lower O_3 level.

J. A. Graham: That assumes that the mechanism is the same at the high concentration. In the modeling by Miller [Chapter 29 of this volume], the tissue dose of O_3 was found to be dependent upon concentration: at higher doses the O_3 molecule may hit the pulmonary tissue; at lower doses, the molecule may not hit the tissue.

S. V. Dawson: I absolutely agree with you.

J. A. Graham: My last question is: When you refer to macrophage motility, are you talking about chemotaxis?

S. V. Dawson: Yes. This is a standard migration test that Les Schwartz has published.

J. A. Graham: For O_3 in the monkey, why should that parameter--chemotaxis--be any more sensitive than all the parameters already studied and reported relative to the macrophages?

S. V. Dawson: Well, I don't think it's likely to be a more sensitive parameter, but it ties to the mechanism of damage. It tests the hypothesis that the respiratory bronchiole becomes tied up by all of this. One could argue, perhaps, that finding an effect in that area at 0.2 ppm O_3 supports that hypothesis.

J. A. Graham: Those studies aren't going to be done on humans. In terms of trying to correlate the epidemiologic, human clinical, and animal studies, I don't see how macrophage chemotaxis directly relates to humans.

S. V. Dawson: Well, the monkey is as close as we can get. It's hard to do that morphometry on the human.

P. E. Morrow: In what species do you plan to do the pentane exhalation study?

S. V. Dawson: Humans.

P. E. Morrow: How do you propose to do that, inasmuch as the dietary component (lipid diet) and also the level of oxidant are so significant in what you would apparently measure? The active level of oxidant breathed and dietary component of lipid affect the breakdown. How do you deal with that in the human population?

S. V. Dawson: When a subject is exposed to O_3, there are really two different aspects. First, if the subject comes from a polluted area, does he produce more pentane, reflecting more oxidant damage to his lungs? Secondly, when given O_3, does he produce more pentane before or after the experiment?

Comment: As I understand it, the use of pentane and isopentane and the relationship between them can help overcome some of the issues of metabolic production in the background. Ethane has also been used.

Comment: With pentane, the method of detection is much more precise. The ongoing levels have to be active; there's no "memory" of what the individual breathed yesterday.

Question: There is some published work on pentane and ethane exploration with presumably oxidizing cytotoxins but not respiratory pollutants. Wouldn't it be useful to have some animal work on this as well?

S. V. Dawson: Yes, that's absolutely valid and we will explore it. One of the suggestions is that we might do such work at UCD.

Question: In the walk-in chest complaints study, how do you plan to correct for the self-selection bias? Secondly, what kinds of objective measurements do you plan to analyze for? What are the end points?

Comment: Of necessity, we will use the somewhat gross end point of a self-selected complaint. The frequency of such complaints in areas of widely differing pollution levels and yet subject to the same barrage of publicity about the episodes was thought to be a way to look at real effect. We have already done some feasibility studies on emergency room visits; the study could probably be more finely tuned by the use of data on respiratory therapy services or the medications administered to people coming there. (We exclude nonrespiratory complaints.) The concept is to set up a network and have it in place so that, when acute health advisory levels are reached, the frequency of visits during those periods can be compared to other seasons or years.

Question: Where [in Table 39-1] is the leukocyte function test to which you referred?

S. V. Dawson: I don't know. That's tentative; we're just exploring it as a pilot study that might possibly be funded.

A. P. Altshuller: Could you or Dr. Hackney expand somewhat on the directions the human experimental work will take beyond what has been done to date?

Comment: As far as we know, all previous work has been on very highly self-selected individuals--laboratory technicians, college students, etc.--that were nonrepresentative of general populations. In our case, we certainly don't have any means of forcing people from the general population to go into the study, but at least we are sending out letters asking the general population, individual by individual, to join our study. That, as far as I know, is completely new. Also, I think that the 0.3 ppm level is well worth getting additional data on.

A. P. Altshuller: The study will be conducted at that level only?

Comment: Yes, that's the plan at least for the first year.

J. D. Hackney: The point I would make is the following: The epidemiologists will present us with some subjects who are asserted to be sensitive to photochemical pollutants in view of a more rapid decrement in forced expiratory volume over five years. These subjects will be compared with subjects showing almost no decrement. It may be that the subjects showing a very large decrement are also sensitive to O_3 over a short-term 2-h exposure; such results would be exciting. There would be at least two possible explanations: (1) the O_3 somehow caused the rapid decrement; (2) having a decrement is itself a predisposing factor for sensitivity to short-term O_3 exposure. Either one of those explanations would be of interest. That's the positive side.

On the negative side, being able to do the study will depend on Dr. Detels' identification of these people. He already has them but the question is whether they will volunteer in sufficient numbers.

Dr. Detels would do all the matching. He would send us the subjects and we would test them. At the end, we'd try to sort it all out and see if there was some correlation between epidemiologic decrement and short-term clinical response.

40. PANEL DISCUSSION

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Aubrey P. Altshuller
Donald C. Borg
Robert S. Chapman
William Frietsch, III
Marvin Goldman
John R. Holmes
Fred J. Miller
James L. Whittenberger

T. Crocker: Of the possible items of agenda for this Panel Discussion, it is my impression that most of those present favor some sort of scientist-administrator exchange. In my view, such an exchange is potentially very fruitful in that we might receive some information about the character of future scientific review and program selection activities. In a certain sense, we stand at the beginning of a phase of investigative effort. As researchers, we all want this effort to be relevant and meritorious, but we need to understand the intended mechanism for judging the quality and relevance of our work.

To begin, Dr. John Holmes, Director of the Research Section of the California Air Resources Board (ARB), will describe the cooperative agreement with EPA under which ARB will conduct scientific review.

J. R. Holmes: I certainly concur with the point made at the outset of this meeting: It's very important, in selecting and managing research projects of this kind, to bear in mind that we need scientific excellence and regulatory relevance. In developing a program, we must consider both aspects.

In ARB, we have developed a joint committee consisting of members from the ARB Research Advisory Committee as well as from an Air Quality Advisory Committee of the California State Department of Health. These individuals are scientists who are also involved (either peripherally or directly) in making recommendations that serve as a basis for air quality standards. In my opinion, the panel did an outstanding job in assembling a package of studies that are both scientifically excellent and relevant to what we view as the problems in setting an oxidant standard both nationally and in California. At this point, none of the individual studies are "cast in stone"; there is still time to hone and polish and make them even more relevant and scientifically sound. The investigators and certainly ARB are very interested in any comments from this group; we've received a number already.

In my opinion, before seeing the project through to completion, we need to set up a system of scientific guidance that differs from the systems currently used by EPA and ARB. In the management portion of our proposal to EPA, we outlined plans for a joint technical committee to consist of representatives from the State of California (2), EPA (2), the National Laboratories (1), and the scientific community at large (2). This committee is large enough to incorporate a number of different disciplines and yet small enough to permit efficient discussions and limited travel cost. I think this approach will work out well. But I would appreciate any comments or additional guidance on how technical review of the project should be carried out.

W. Frietsch: We in EPA also have a review group that is looking at this project (as well as others) strictly from a planning viewpoint [see Chapters 2 and 3 of this volume].

T. Crocker: My understanding is that Dr. Paul Altshuller is involved with a separate Oxidant Research Committee within EPA's general approach to the criteria air pollutants. This Committee develops strategy plans and documents and will be responsible, as I understand it, for a mission statement. I may be overstating the level of supervision that the Committee will undertake, but I know that Dr. Altshuller has given some thought to potential developments and to the level of guidance that may be forthcoming from this Committee. Would you care to speak to this point, Dr. Altshuller?

A. P. Altshuller: Well, perhaps a bit of explanation is in order. During congressional deliberations several years ago, the problem of the relevancy and responsiveness of EPA in-house research to the regulatory needs of the Agency came to a head. A fairly drastic change in the distribution of resources was under consideration; the Deputy Administrator asked that we be given a year to look into alternatives. We proposed a pilot Research Committee program; our proposal was accepted. The pilot program was gradually expanded and today includes essentially all of EPA's research; the Oxidant Research Committee is just 1 of 16 Committees covering air, water, radiation, pesticides, etc.

The individual Committees have levels of responsibility for resources ranging from $10 million to $60 million. The Oxidant Research Committee's responsibility is in the ~$30 million range. Obviously, the Committee cannot (and was not designed to) perform in-depth peer review for each small segment of a $30-million program. Rather, the purpose of each Research Committee is to interface between those responsible for research and those responsible for regulatory development and enforcement. Thus, each Committee is largely composed of individuals with technical backgrounds: personnel from EPA's regulatory divisions, from the Office of Enforcement, from regional offices, and from the Office of Research and Development (ORD). Each Committee is co-chaired by a representative from ORD (in the case of the Oxidant Research Committee, Ken Berry) and a representative from the lead program office. For oxidant research, the lead program office is the Office of Air Quality Planning and Standards.

The Committees serve as a mechanism for expression of concerns about scientific relevance, responsiveness, and the timeliness with which outputs from various research programs become available. The obvious question is: How can we assure scientific quality? In part, this depends on the competence and the dedication of the technical personnel within EPA. It also depends on a peer review mechanism. Dr. Steven Gage, Assistant Administrator for

Research and Development, has been very concerned about the peer review mechanism for both in-house and external research. We are revising the research grant program to return to a peer review procedure resembling that of the National Institutes of Health (NIH) but which will also include a relevancy review. At present, we don't have all the answers on how to integrate these two aspects, but both are vital. Regardless of whether a research program involves pass-through money in another agency, agency agreements, contracts, or what have you, the same general question--how to achieve a scientific product that will be accepted by the scientific community and also usable in criteria and standards development--remains a vital issue.

Question: Has any significant peer review mechanism been set up?

A. P. Altshuller: A specific mechanism for research grant peer review is being set up. It will consist of a group of four committees that will periodically review grant proposals for both scientific acceptability and relevancy. Because of certain congressional and other limitations on the movement of funds, we have a number of "pigeonholes" where we have to "put the money," so to speak, and we will fill these up with proposals until the funding limit is reached. To do this, we'll work down a list of projects scored for merit as well as relevancy. In a sense, it's a tripartite system: scientific merit, relevancy, and the amount of money available for the subcategory.

Comment: You still haven't answered the question.

A. P. Altshuller: Would you care to enlarge on your question?

Question: Aside from research grants, is any peer review system under consideration?

T. Crocker: Dr. Altshuller, I believe the question refers to the oxidant program that is the subject of this workshop. You're talking about an extramural grant review program that may not be related to our particular focus.

A. P. Altshuller: Well, it would be related to all grants, including those concerned with oxidant air pollutants. We're just working on possible mechanisms for review. A question which does come up is: To what extent can the other body of expertise available to EPA on a continuing basis--namely, the Science Advisory Board (SAB) and its subcommittees--participate? Just last week, we had some discussions with the Director of SAB, Richard Dowd; we discussed some preliminary proposals to involve the Clean Air Scientific Advisory Committee in more in-depth review of criteria pollutant research, with particular emphasis on oxidants. The question is: Where do we go from there, when we get down to the question of reviewing program components that represent a million, half a million, or a quarter of a million dollars? This would demand large numbers of people who are willing to commit some significant fraction of their time. Very likely we would end up with well over 100 individuals just in the area of health. These 100 individuals would have to be willing to commit a certain fraction of their time to interact in peer review of a wide variety of projects in health, modeling, ecosystems, control technology, etc. And this has not been worked out.

Comment: The details of the review system have all been set down by Dr. Gage. If you're interested in obtaining those details, I suggest that you contact Dr. Marlin at EPA Headquarters.

A. P. Altshuller: Having talked quite a bit with Dr. Marlin, I'm not sure that the array of detailed information is really there. The mechanics may be there for the research grants and for the cooperative agreements. As for the various other categories, it is probably a little premature to say that we have all the answers.

F. J. Miller: SAB will review the EPA in-house projects on March 20. SAB will also perform scientific review of the Department of Energy (DOE) transferred projects. So mechanisms have been set up. If your question was whether the DOE-EPA projects will be reviewed, the answer is "Yes."

D. C. Borg: With regard to Dr. Altshuller's point, it does take a lot of people to carry out peer review (a la NIH, in any case). What's more, as thorough and reassuring as that sort of review may be, there are certain disadvantages. It grinds with extreme slowness, to say the very least. Other agencies (perhaps less well known to this audience) carry out peer review in a somewhat different fashion. For years, the National Science Foundation (NSF) has proceeded with peer review (in the physical sciences) much as do editors for peer review journals: namely, asking for outside written opinions and then presenting a consensus of that opinion to an internal review board. In the life sciences area, there are also panels. Those who have served on the NSF panels know that they don't have as much time to spend on an individual grant application as does an NIH study section. The NSF panels perform something of an intermediary function: they are much more advisory than the NIH study sections. In the physical sciences, however, NSF has managed to do a fairly creditable job without all of that. Thus, I would remind EPA that the NIH way is not the "only" way to achieve peer review.

A. P. Altshuller: Yours is a rather interesting comment, for this reason: The EPA research grant/cooperative agreement review system that was in place from 1973 until 1979 was very similar to the NSF system. Extramural reviewers were asked to individually review each project and to submit written comments. A single in-house scientific reviewer also assessed the project. A judgment was made by technical management within the individual Laboratory. (Later, that judgment added up to whether or not the project was funded.) It's precisely this sort of system that was criticized, resulting in a return to an NIH-type system.

One of the principle criticisms was that we were involving too few investigators. Critics complained that we tended to be satisfied--once we had found a group of investigators who could do the job in a specific discipline--to "ride along" with those investigators in other disciplines. I'm not sure that was a valid criticism; in all honesty, I don't know where the additional investigators would have been. As far as I could discover, we had sufficient resources to fund essentially all of the competent investigators. In some areas it may have been a specious criticism; in others, it may have been a relevant criticism.

M. Goldman: As someone who is not overly familiar with the EPA system of review, I've still not received an answer for a question I jotted down on my way to this workshop: How do you equitably separate the problem of relevancy (which I assume to be an intra-agency one) from that of scientific merit? At this workshop, I hear what seems to be a plea for an equitable peer review system that will take care of both the intra- and extramural programs regardless of how they are handled administratively. In other words, the science--regardless of who's doing it--should be equally good, and a mechanism for assuring this must exist. This relates, perhaps, to a mechanism to provide continuity of support for longer-term programs. If high-quality science is to be attracted to these programs, the issues of peer review and of how information will be delivered to the various clients are important parts of the overall spectrum. From what I've heard here, progress is being made but the system is not yet complete. This business of "extramural versus intramural program methods" leaves me and others with some confusion.

T. Crocker: Your comments fairly accurately summarize the situation in terms of the structure and plan for Agency supervision. It's moving but it hasn't jelled.

With respect to extramural grant review, Dr. Whittenberger chairs a special SAB Subcommittee that has been involved in [the redirection of Theme 1 of the Energy Health Effects program; see Chapter 3 of this volume]. That body, of course, serves at the request of EPA; still, in the sense that it consists of extramural scientists, the Subcommittee provides something of an extramural peer review function. Perhaps Dr. Whittenberger would comment on possible expansion of the Subcommittee's mission to meet some of the issues that have been raised.

J. L. Whittenberger: Our Subcommittee was not asked to consider scientific merit. We were asked to assist in the planning process in cooperation with EPA scientists and administrators. We have raised questions about scientific merit but do not consider it our responsibility to exercise the kind of judgment that we would perhaps like to exercise. We're not constituted to do that. In our last meeting, we did discuss the need for peer review for scientific merit as well as for relevance, and suggested that SAB might take responsibility for setting up subcommittees that would be more properly constituted to carry out such peer review. This discussion occurred at the end of our meeting; we have taken no action to implement it.

I think it would be worthwhile to respond to Dr. Alpen's comments [Chapter 6 of this volume] and to some of the comments by Dr. Goldman illustrating the need for a much better understanding, on the part of scientists outside EPA, of the constraints under which EPA operates as a regulatory agency. Dr. Altshuller mentioned some of the items on my checklist; I'll touch on some that he didn't mention. These comments will express my point of view as an individual, not as an SAB representative.

With regard to Dr. Alpen's comments [Chapter 6 of this volume], when I wear my "Harvard hat" I agree with everything he says about toxicology and the relevance of different kinds of studies in respect to toxicologic analysis of any obnoxious compound. When I put on my "EPA hat," however, I am forced to adopt a very different concept of what is "relevant." One must never forget that research conducted within a regulatory agency is very different from research conducted outside a regulatory agency. EPA's research program is driven by program offices: primarily, the Office of Air, Noise and Radiation; the Office of Water and Waste Management; and the Office of Toxic Substances. When EPA was established, research and development were put in one office that was supposed to respond to the program needs of other parts of the Agency. This arrangement has continued to present certain problems.

We must remember that the program offices are driven by the specific requirements of legislation. Sometimes EPA is undeservedly blamed for situations for which it is not responsible. The fact that scrubbers are required in power plants that can use low-sulfur coal does not reflect perversity on the part of EPA but, rather, legislation that was written so as to keep people employed in Appalachia. Another example is the Toxic Substances Control Act, an act that many consider almost impossible to administer.

Several years ago, the program offices complained about ORD research: they said that the scientists wanted to "do their thing" without responding to the needs of the program offices. Congress became upset and, as Dr. Altshuller said, demanded that something be done. Dr. Gage set up a task force to examine how other agencies handle this problem. The end result was the creation of the Research Committees.

The Research Committees have certain advantages and certain limitations. One problem is the need to support long-term basic research as well as more immediate regulatory-related research (i.e., research to provide certain "answers" within six months or a year). Somehow, the long-term research needs seem to "get lost" before they are ever funded. This is due not only to limitations of the Research Committee approach but also to limitations inherent in the legislative process. Congress recognizes EPA's need for basic research; the Authorization Subcommittee encouraged Dr. Gage to plan a so-called Anticipatory Research Program. But Dr. Gage has had trouble obtaining funding for that program because the Appropriations Committee doesn't look at things the way the Authorization Subcommittee does.

Dr. Gage has been able to set up so-called Centers of Excellence in universities or consortia of universities. In designating a Center of Excellence, EPA adds some relatively long-term funding to orient an already strong program to EPA needs.

On the subject that is paramount in this Panel Discussion--peer review--I'd like to supplement some of the previous comments on EPA's "credibility problem." From Mr. Costle on down, there is a great deal of concern within EPA about peer review of all research activities (both intra- and extramural). As Dr. Altshuller said, EPA originally had a peer review mechanism which worked well in many parts of the Agency. It did not work as well in other parts of the Agency, and those places may have had more influence on Dr. Gage. Thus, he made the decision to set up the centrally administered Grants Program. This program, which is spelled out in the Federal Register, is now getting underway. The first committee meeting is scheduled for next month and the notifications will be made, I believe, in April.

That takes care of a certain part of EPA-funded research, but what about the cooperative agreements, contracts, and so on? I don't believe those mechanisms have really been developed yet, although I know that Dr. Gage is very interested in putting such systems in place.

Some of the problems that I've referred to very briefly were the subject of a study by the National Academy of Sciences reported in 1977. In 1978-9, I participated in a study, mandated by Congress, in which we looked at EPA health effects research from the point of view of relevance to program needs as well as quality. After receiving our report, Mr. Costle asked the Environmental Health Advisory Committee (an SAB committee) to assume an ongoing responsibility for peer review of EPA's major health effects laboratories. This will encompass the intramural and extramural research that is not covered by the centrally administered Grants Program.

I'd be glad to answer any questions about peer review.

R. S. Chapman: I'm concerned, because the essential focus of workshops such as this, and of peer review generally, tends to be the review of scientists within EPA by scientists outside EPA. For two reasons, I think that such a focus is not very effective in resolving limitations in EPA's scientific credibility. First, there is already substantial agreement between intramural and extramural scientists on the standards required to ensure scientific merit in research projects. Second, instead of this constant droning about review of scientists by scientists, what we need is an educative function by scientists both within and without EPA to all levels of the administrative and legislative structure under which we operate. Such a function, if given "teeth" and if perpetuated, would be far more useful than this constant reshuffling of various ways to have scientists critique the work of other scientists, which just isn't "getting the baby washed," to be perfectly honest with you. In my opinion, we are today under the same administrative and legislative pressures to do work that's against our better judgment as we have been ever since EPA was formed. And so I believe that education "up the line" will be a lot more useful and fruitful than yet another mechanism by which scientists can review scientists. Within the Agency, we're quite inclined to agree with scientists who criticize the merits of our work; we tend to say, "Yes, we agree with you." When our critiques are carried into the administrative area, however, our better judgment tends to be paired against the conflicting pressure of "The mandate is here now, the money is here now, the money won't be here if you don't think the work can be done now." The former pressure always loses out to the latter. Until the former pressure has more authority, I think that this kind of exercise is, to a considerable extent, rather futile.

J. L. Whittenberger: I understand your feelings and I agree that the program of which you are a part was subjected to a very critical and in many ways unfair review. Personally, I felt that the CHESS program (one of the programs that took place over a number of years in the unit to which Dr. Chapman belongs) was a very important program; it should have been constructively criticized but continued. One of the reasons we don't today know more about the effects of oxidants on the health of people in Los Angeles is the dismantling of that program.

On the other hand, I agree strongly with Dr. Goldman that scientists anywhere ought to be subject to critical review by their peers. In visiting many EPA laboratories, I have encountered an almost uniform desire to be critically reviewed. Many scientists feel that their work has to stand the test of peer review not only for publication in scientific journals but also for introduction as evidence in court. They want that evidence to stand up. So, I hope you'll be patient with the attempts to extend consistently applied peer review within EPA.

R. S. Chapman: I agree completely. I'm certainly not in favor of less peer review for EPA scientists. I'm in favor of widening the perspective of discussions like this (which, I might say, always come at the end of a meeting instead of at the beginning or middle) to consider perhaps two more aspects: (1) the entry of peer review at a higher administrative level, and (2) giving the working scientists some tools for meaningful implementation of their ideas coupled with the ideas of the peer reviewers. I'm certainly not arguing for less peer review and scientist-to-scientist contact; rather, I'm arguing for more effective use of it.

J. L. Whittenberger: I certainly agree. I encountered that same feeling in many of the EPA laboratories. The scientists desire far more feedback, both from SAB and from the scientists who reviewed their work.

T. Crocker: I think what Dr. Chapman is advocating might go even to the level of peer review at upper administrative levels of the agencies and of the Office of Management and Budget. In other words, who tells those gentlemen how much to assign to a specific project in a specific year?

J. L. Whittenberger: I would extend your comment to Congress as well.

T. Crocker: In other words, it doesn't do us "bench people" a lot of good to chew at each other's quality when the decisions about relevance and resources are made far beyond our reach. I think Dr. Chapman is asking how we might reach that level. Possibly, increased communication between administrators

and scientists would help. But high-level administrators are not present at this workshop.

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